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STUDIES ON THE CLADOSPORIUM BLIGHT OF SWEET PEA¹

By H. M. GOOD²

Abstract

Following the appearance of the *Cladosporium* blight of sweet pea in Ontario in 1945, a study of the causal organism and the disease was carried out. As a result of this study the name *Cladosporium album* Dowson has been retained for the organism in preference to *Erostrothea multiformis* Martin and Charles, or *Hyalodendron album* (Dows.) Diddens. Temperature and humidity studies have indicated that the parasite and the disease develop most rapidly at 25° C., and that high humidity is necessary for infection and for fruiting on the leaf. Penetration is stomatal, without appressoria, and occurred 10 times as often into leaves with 'open' as into leaves with 'closed' stomata. Evidence that *C. album* may show a positive hydrotropism was obtained. Frequency of penetration increased with an increasing gradient around the stomata, and this was interpreted as evidence that hydrotropism is a factor in penetration. Host range studies and a comparison of frequency of penetration into an immune and a susceptible species showed frequent penetrations into the immune species but no appreciable development in any but sweet pea.

Introduction

In July of 1945 a blight of the sweet pea, *Lathyrus odoratus* L., caused by *Cladosporium album* Dowson, was found for the first time in Ontario. Although this disease has been reported from a number of widely separated places, no detailed study has been published, and it was felt that its potentialities should be more fully investigated. In the course of the study, the fungus and host were found to be favourable material for an attack on certain general problems of stomatal penetration, and special emphasis has been laid on these.

Cladosporium album was first described by Dowson in England in 1924 (6). Subsequently it has been found in the United States, Europe, Western Canada, and East Africa. Losses from the disease have not usually been considered significant, though one report suggests otherwise. In the outbreak that provided source material for this study, the flowers were produced fairly normally, but the injury to the leaves was considerable, and must have caused a significant decrease in yield.

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. An abridgment of a thesis presented in May, 1947, to the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

² Lecturer.

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Symptomatology

The conspicuous feature of the disease is a blotching of the leaves with circular to irregular buff areas. These appear first as small yellowish flecks, already becoming necrotic, which spread slowly without forming any definite limiting margin. Single lesions are rarely more than 1 cm. in diameter though several may coalesce to involve most of a leaf. Fig. 1 shows two leaves with lesions of different ages and extents. Similar lesions may occur on the stem.

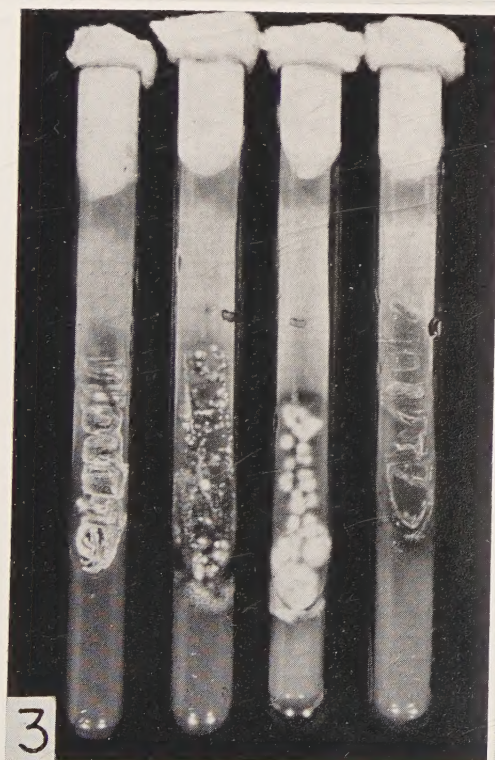
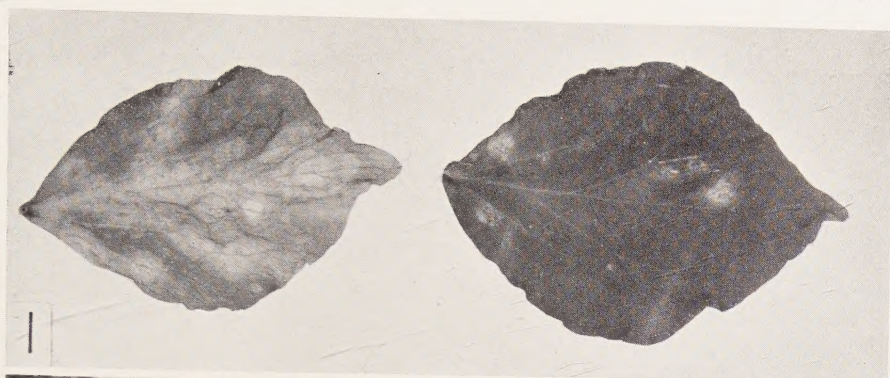
The length of time taken for the lesions to become macroscopically visible varies greatly with the conditions. In a moist chamber in the greenhouse at a temperature of 25° C., flecks appeared in four to five days, while at 8° C. appearance was delayed for two weeks. High humidity had a slightly accelerating effect on the rate of disease development. Sporulation begins soon after the onset of necrosis of the leaf cells and a powdery layer of white conidiophores and conidia is formed over the buff area of the lesion on both sides of the leaf. Some conidiophores may arise from the green tissues surrounding the necrotic region, but these are never numerous. Often there is little sporulation, particularly on plants grown under winter conditions in the greenhouse. On these, small necrotic areas with few conidiophores were all that appeared when the plants were held several weeks after inoculation.

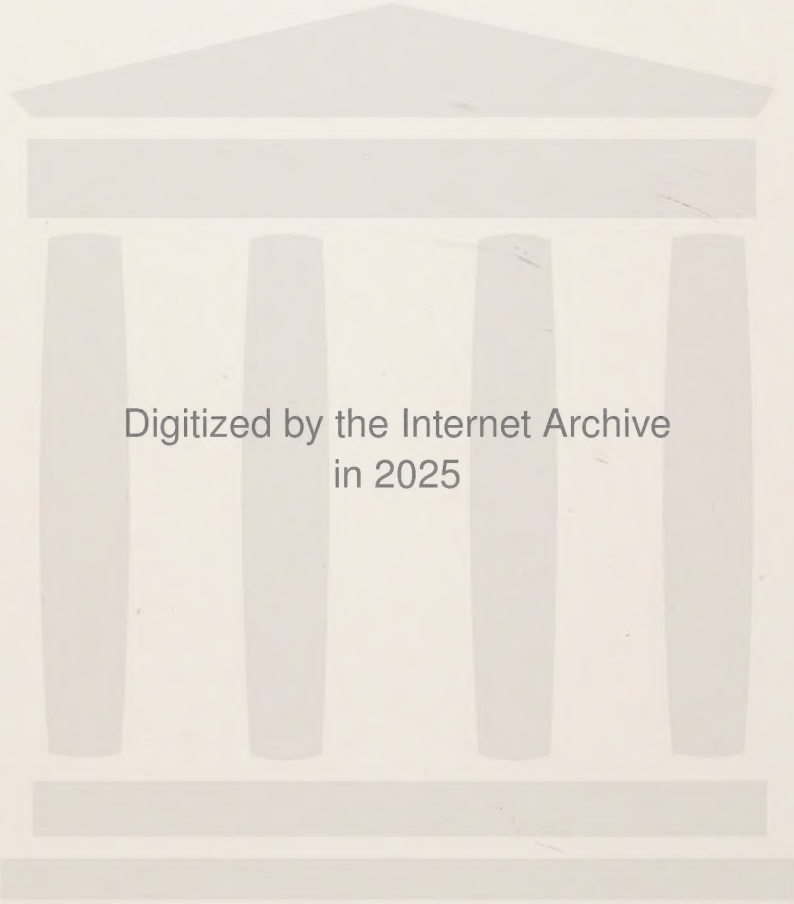
The development of the disease is more limited in seedling leaves than in adult, partly because most infected seedlings drop their leaves within 10 days or two weeks after inoculation. Dropped leaves showed almost no sporulation, even when placed in a moist chamber immediately after falling. Fruiting of *C. album* was light under greenhouse conditions in Toronto.

Both spring-flowering and summer-flowering types of sweet pea were attacked in the source outbreak. The spring-flowering ones appeared more seriously damaged but since they were well past their best season when first seen, little significance can be attached to this appearance. In greenhouse tests on seedling plants no difference in reaction was evident.

This picture of the symptoms agrees closely with Dowson's observations (6) except for the poor sporulation, and the dropping of seedling leaves before the lesions had much more than appeared. Dowson found good sporulation on most of the infected leaves, and defoliation only with leaves with large necrotic areas. All the work reported here has been done under glass in Toronto where conditions for growing sweet peas are not ideal. The plants have been significantly less robust than those pictured in Dowson's report and it is thought that this is sufficient to explain any differences noted. A tendency for spindling plants, grown in winter under very low light intensity, to show least sporulation and earliest defoliation, supports this view.

-
- FIG. 1. Leaves of sweet pea showing lesions caused by *Cladosporium album*.
FIG. 2. Microconidia of *C. album* on submerged mycelium from a cornmeal agar slant. $\times 1000$
FIG. 3. Types of cultures of *C. album*. From left to right strains (a), (b), (c), and (a).
FIG. 4. Microsclerotium of *C. album* submerged in cornmeal agar. $\times 750$





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Isolation and Study in Pure Culture

The fungus was isolated directly from the diseased leaves using a dry needle. In most cases this resulted in a pure culture uncontaminated by bacteria. Some single-spore culturing was done but the bulk of the transfers were of mass material.

Study of the fungus on a wide range of media showed that the type of colony varied considerably depending on the substrate. On cornmeal agar, a powdery layer of conidiophores, with little suggestion of felting, was obtained. This type of colony also formed on Czapek's and Barnes's agars. However, on potato dextrose, malt, prune, pea, and nutrient agars, a raised and felted colony developed with large quantities of sterile mycelium. The colonies rarely became more than 2 cm. across. From the agars tested, cornmeal was finally adopted for most of the studies since it gave a considerable yield of spores and a type of colony with little sterile mycelium. This was an advantage in studying the morphology of the fungus. Moreover, the work of Martin and Charles on *Erostrotheca multiformis* (18) was done on cornmeal and it was desirable to use the same medium as these authors. In cases where the extent of growth was to be measured, either prune or potato dextrose agar was used.

The influence of temperature on the development of the fungus in culture was studied with reference to both the growth of colonies for a number of days and the growth of germ tubes for a 48 hr. period. The fungus developed normally within the range of 5° C. to 30° C. Some development took place at temperatures lower than 5° C., but growth was extremely slow. At temperatures higher than 30° C., germination occurred, but the fungus was not able to continue beyond this stage. The optimum temperature lay in the vicinity of 25° C.

Cladosporium album was found to be tolerant to considerable range in pH. Using both colony development and germ tube growth as criteria, good growth was obtained within the range of pH 4 to pH 8 with a maximum rate at pH 6.

The effect of relative humidity on growth and sporulation in culture was studied using a method adapted from Hopp (15). A stream of air was passed through a saturated solution of a salt to give a definite relative humidity, and then was directed onto the surface of an agar slant on which spores of *C. album* had been placed. Humidities of 45, 65, 80, 90, and 100% were obtained using calcium chloride, sodium nitrite, ammonium chloride, sodium carbonate, and water. In order to maintain the substrate at a constant hydration, the tubes containing the slants were pierced with two holes and were placed in a water-bath so that water lost from the upper surface could be replaced from below. Under these conditions, relative humidity had no effect on the type of growth, or time or extent, of sporulation. This was interpreted as evidence that the fungus is capable of transporting water along the conidiophores and spore chains rapidly enough to support growth at all humidities tested, provided the supply of water to the absorbing organs is adequate. Further

mention of these results will be made in connection with the influence of relative humidity on disease development.

A tendency for *C. album* to be somewhat unstable in culture was noted during the investigation. No aberrant types of cultures were seen for the first few months, but at the end of six months a number of different types had appeared. These were purified by transfer and by single spore isolation and ran true to type in all cultures but one. In this manner three distinct types, illustrated in Fig. 3, were obtained: (a) powdery white type slowly forming dark mycelium in the substrate; (b) powdery white type forming dark mycelium and microsclerotia in a few weeks; and (c) fluffy white type slow to form dark mycelium, which usually forms in masses rather than in microsclerotia. This last type was sterile and resembled a culture obtained from the Centraalbureau voor Schimmelcultures derived from a collection of Dowson's. In infection experiments there was no difference in pathogenicity between these various forms except that the sterile forms did not fruit normally nor did they form substomatal knots.

Morphology and Taxonomy

Various reports of the white blight of sweet pea have dealt differently with the taxonomy of the causal organism. When first describing the fungus in 1924, Dowson (6) decided that it should be considered as a *Cladosporium* though it was entirely hyaline. In 1928, Martin and Charles (18) accepted the name *C. album* and reported that they had obtained the perfect stage for which they created the new genus and species, *Erostrotheca multiformis*. This ascomycete was said to form a number of types of spore in culture, viz. *Hormodendron*, *Ovularia*, *Haplaria*, *Pseudofumago*, and *Pseudosaccharomyces*. In 1934, Diddens (5) transferred *C. album* to the genus *Hyalodendron*, which he had founded upon wood-pulp fungi from Sweden. In view of these reports, special attention was given to the morphology and taxonomy of the fungus.

The principal type of spore found was the *Cladosporium*. These spores were borne in branched chains (Fig. 7) and were mostly one-celled and oval though a few long, cylindrical, two-celled ones occurred as basal elements of the chains. The *Cladosporium* spores ranged in length from 3 to 16μ and in width from 3 to 5μ .

A second type of spore was also found though not with regularity. These were small ($1 - 1.5 \times 2 - 5\mu$) and were borne singly or in agglutinated groups, usually on branched conidiophores (Figs. 2 and 6). They occurred submerged in the medium and only in cultures approximately one month old, apparently always associated with dark microsclerotia (Figs. 4 and 5) typical of the 'b' strain described above. The other strains showed neither microconidia nor distinct microsclerotia though masses of dark mycelium sometimes formed.

These microconidia and microsclerotia suggest spermatia and perithecial initials of an ascomycete. However, no signs of the perfect stage appeared even when mixtures of all the available cultures were made. It is possible

that *C. album* is an ascomycete that is undergoing loss of functional sexuality or that it is heterothallic and only one strain was available. Unfortunately no decision could be reached on this point. Attempts to get cultures from various parts of the world, where *C. album* occurs fairly regularly, have so far been unsuccessful.

No further noteworthy structures were found in the Ontario material of *C. album* though a suggestion of chlamydospore formation was observed in some of the older cultures and some germinations resembling budding were seen. These suggest the *Pseudofumago* and *Pseudosaccharomyces* forms of

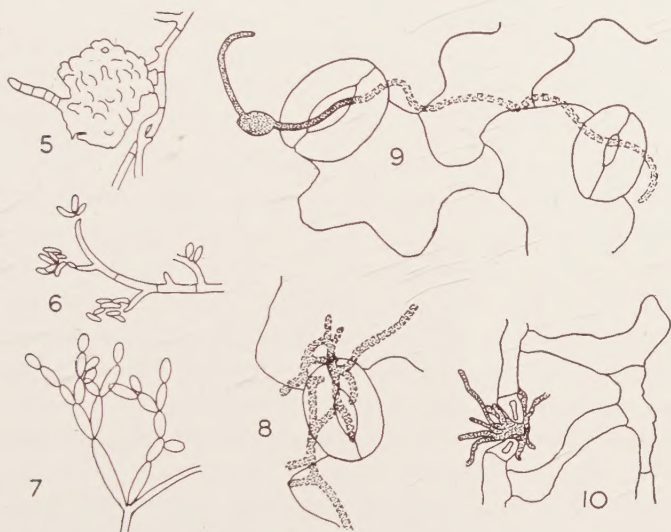


FIG. 5. *Microsclerotium* of *C. album*. $\times 370$

FIG. 6. *Microconidia* of *C. album*. $\times 580$

FIG. 7. *Macroconidia* of *C. album*. $\times 370$

FIG. 8. *Initiation of a substomatal knot just prior to fruiting*. $\times 580$

FIG. 9. *Penetration of C. album through stoma of sweet pea*. $\times 580$

FIG. 10. *C. album fruiting on leaf of sweet pea*. $\times 350$

E. multiformis. However, since Martin and Charles state that in certain of these forms the transitions can be easily understood and interpreted from a single slide and no such distinct combinations were found in the Ontario collections of *C. album*, it is thought that these occasional occurrences cannot represent the *Pseudofumago* and *Pseudosaccharomyces* of *E. multiformis*.

A comparison of the observations on the Ontario collection of *C. album* with those of Martin and Charles on *E. multiformis* shows that while a number of similarities exist, the range of imperfect spore forms can only be made to agree by considering certain unusual growth forms as spore types. The *Hormodendron* type exists in the Ontario material but not as a form distinct from the *Cladosporium*. The microconidia resemble the *Ovularia* and *Haplaria* stages of Martin and Charles but there are not two distinct types. They

agree more closely with the description of *Haplaria* though they did not develop nearly so quickly, as was reported for *Erostrotheca*. No regular occurrences of the *Pseudofumago* and *Pseudosaccharomyces* stages were found and neither of these stages was sufficiently clearly marked to be considered as a spore type.

In addition to what are considered as differences in the spore forms occurring in the Ontario collection of *C. album* and those found in cultures of *E. multiformis*, one other consideration should be made regarding the connection of *C. album* and *E. multiformis*. In Martin and Charles' paper, the cultures of *E. multiformis* are shown as widespread and zonate, approximately covering a Petri dish. In both general appearance and extent of growth, these differ radically from those found characteristic of any strain of the Ontario collection and from those described by Dowson. Considering these differences and the lack of definite agreement in the morphology in culture, it is felt that there is some doubt that *E. multiformis* is the perfect stage of *C. album*.

Diddens' removal of *C. album* from the genus *Cladosporium* brings up the question of the proper name for the imperfect form. Apart from colour, the sweet pea fungus shows close resemblance to other Cladosporia and the question is whether colour alone is a sufficient basis for excluding the fungus from that genus. Dowson gave considerable attention to this problem and decided that the fungus could be properly called a *Cladosporium*, the close resemblance to *C. herbarum* was noted by Brooks (6) and in parallel cultures with *C. fulvum*, no difference could be made out until after the onset of fruiting in both forms. This and the fact that *C. fulvum* mutates to a white form indicates that colour is of little fundamental importance in determining taxonomic relationships. While it is realized that the present classification of the Imperfects is an artificial one, based to a large extent on such factors as colour, it is felt that in the case of the sweet pea fungus, the name *C. album* is more indicative of the type of fungus concerned than is *Hyalodendron album* and probably indicates as much actual relationship. The name *C. album* Dowson has therefore been retained.

The Influence of Environment on Disease Development

Temperature

A series of air temperatures was obtained by covering the soil cans of Wisconsin temperature tanks with perforated cellophane. The temperature of the air in the cans agreed closely with that of the water in the tanks. Plants used in these experiments were removed from the soil and their roots placed in normal Pfeffer's solution to avoid the high carbon dioxide production of the soil in the rather poorly ventilated chambers. The leaves were inoculated by brushing a suspension of spores over the surface and the plants were placed in cans with moist paper towels around the sides to give high humidities. Leaves were removed, cleared, and examined at the ends of two, four, and five days,

and later checks were made at 10 days and three weeks at the lower temperatures. The examinations provided a picture of the penetration and development at various temperatures.

At 35° C. a few spores had germinated at the end of two days. However, no further growth took place and the leaves died in a few days. These observations agree with those on temperature effects in culture in indicating that the fungus is unable to develop appreciably at 35° C.

At 30° C. germination was good after 48 hr., but the germ tubes were short and no penetrations were seen. After four days the amount of growth had increased but there were still no penetrations. Distinct indication of the disease did not appear till 10 days after inoculation.

At 25° C. growth was extensive after two days, and penetrations were noted. Macroscopic symptoms of the disease appeared in five days. A slightly slower development took place at 20°, but very minute flecks showed on the fifth day.

At 15° C. most of the spores had germinated after two days, but the germ tubes were short. However, within four days penetration had occurred, and macroscopic symptoms appeared in six days. At 8° signs of germination appeared in two days, but it was three weeks before definite flecks appeared.

The disease was found to develop within the range of 8° to 30° with an optimum at 25° C. The close parallel between the effect of temperature on the development of the disease and on the growth of the parasite suggests that in influencing the rate of development of the disease, temperature is acting principally upon the parasite.

Humidity

On glass slides and on leaves in air chambers with good circulation, spores of *C. album* did not germinate at relative humidities lower than 95%, but on leaves in stagnant air, good germination was obtained at relative humidity 90%. This is apparently due to the modifying influence of the leaf on the air at its surface (Yarwood and Hazen (24)). A significant amount of water must be lost through the stomata even at humidities as high as 90%.

The influence of humidity on postinfection development was studied by placing plants in bell jars into which air was passed via towers of saturated salt solution to give definite relative humidities. Plants were inoculated and held in saturated air for 48 hr., then placed in the controlled humidities.

It was found that the leaves in air at 100% and 90% developed fruiting lesions in eight days, though the conidiophores were noticeably shorter at 90%. Lesions were clearly marked at all the lower humidities by the 11th day, but no fruiting developed. Apparently development in the leaf is slowed down only slightly by low humidities, but sporulation is prevented below 80% of saturation.

The studies on growth of *C. album* in culture showed that conidia may be produced abundantly in dry air if the substrate is at maximum hydration. The discrepancy in the reaction of *C. album* to low humidity in culture and on

the leaf suggests that the effect of relative humidity on fruiting on the leaf may be primarily an effect on the hydration of the collapsed leaf tissues in which the fungus grows, and that under dry conditions there is not sufficient moisture available for the production of conidiophores.

Host-Parasite Relations

Contrary to Dowson's preliminary report (6), the fungus has been found to penetrate through the stomata (Fig. 9). No exceptions to this rule have been seen in an examination of thousands of cases. Penetration may occur almost as soon as the spore germinates or after the germ tube has grown several millimetres over the surface of the leaf. In many cases a side branch penetrates. No appressoria have been observed.

There is often an increase of the germ tube diameter immediately after the stomatal aperture is passed. This increase is usually only of the order of 20% but thin hyphae may double their diameter. After penetration, the hypha turns sideways and runs subepidermally, usually for the width of two or three epidermal cells though in some cases much farther. The spread through the leaf is by these straight, infrequently branched, runner hyphae. The host cells do not at first show any reaction and signs of collapse of the leaf tissues appear half a dozen or more cells behind the advancing hyphal tip. As the runner hyphae become branched through the mesophyll, the host cells show first a slight shrinking, then complete collapse. In the collapsed cells, the chloroplasts are no longer distinct though the cell retains a suggestion of green colour for a short time. Later the necrotic areas turn brown. The infected part of the leaf collapses following death of the cells and is much thinner than the healthy portion. Fruiting usually occurs from the necrotic area in which the fungus has spread through both spongy and palisade mesophyll. A hyphal knot forms in the substomatal chamber (Fig. 8) from which conidiophores grow out through the stomata (Fig. 10) and bear branched chains of conidia.

Penetration Studies

Materials and Methods

The studies on penetration phenomena have consisted essentially of a comparison of the effects of various conditions on the frequency of penetration. The method of estimating frequency described here remained the same throughout all experiments on the leaf. Most of the series were done on cut stems. It is perhaps desirable to use the whole plant but the branches appeared perfectly normal for the duration of the experiments, and the control methods used could not readily be applied to potted plants.

Leaves were inoculated with a water suspension of spores brushed on the under surface with a camel's hair brush and spread by gentle rubbing with the fingers until an even film was obtained. The leaves were allowed to dry and were then placed in the required conditions. At the end of the time allowed, the leaves were removed, boiled in alcohol to remove the air and chlorophyll, and cleared in lactophenol. When a leaf was to be examined it was mounted

in lactophenol cotton blue. This gave a very clear leaf in which all layers could be made out easily. The spores and mycelium on the leaf stained dark blue, while that in the leaf remained unstained. A large number of microscope fields were then examined and the number of penetrations noted. The same number of fields was examined for each leaf of any one series and the same number of leaves for each environment. The fields were chosen at random along a path that zigzagged back and forth across the leaf in such a way that fields from all parts of the leaf were examined.

Since the areas examined were equal, the number of penetrations counted was taken as a measure of the frequency of penetration. Repeated counts on the same leaf have shown that this method of estimating frequency is accurate to within a few per cent where appreciable numbers of penetrations occur. The variation from leaf to leaf of the same series is considerably greater, but results have been consistent and for the most part have been highly significant statistically. Analysis of the data from one series on the influence of relative humidity on the number of penetrations gave a mean for six leaflets in saturated air of 12.6 ± 1.5 , while in the same series the mean for six leaflets at a relative humidity of 96% was 39.1 ± 5.8 . The difference of these means is five times the standard error of the difference, giving a significance beyond the 1% level. The variation of the individual counts and the mean in the above experiment is typical of the results obtained. Actual counts have therefore been recorded in summary only.

The Influence of Stomatal Aperture on Frequency of Penetration

The influence of stomatal aperture on frequency of penetration was determined by estimating the numbers of penetrations into leaves in which the stomata were either 'open' or 'closed'. The condition of the stomata was determined by making a stripping into absolute alcohol by the method of Loftfield (17), and the numbers of penetration determined as described above. When almost all of the stomata showed no aperture they were classed as closed and when most showed a considerable opening they were classed as open.

It was found that the stomata did not follow the light and dark experience of the plants but tended to show some opening during the night. In moist chambers, this tendency was more pronounced and some remained open for several days in complete darkness. The period of darkness necessary to obtain closure varied with the previous light experience of the plants, presumably due to the resultant changes in reserve food. The stomata could also be induced to close by causing branches to wilt slightly, and some success was had in controlling the aperture of the stomata in epidermal strippings floated on sucrose solutions of various osmotic pressures. By using these various methods of controlling aperture, counts were obtained of the number of penetrations into leaves with closed and open stomata. The results of the experiments conducted are summarized in Table I.

In Experiments 1, 2, and 3, plants were placed in moist chambers in the greenhouse and in the darkroom. After two days the stomata in the dark

TABLE I
THE FREQUENCY OF PENETRATION OF *C. album* INTO LEAVES
WITH 'CLOSED' AND 'OPEN' STOMATA

Experiment No.	Total leaflets examined	Total fields examined	Penetrations 'open stomata'	Penetrations 'closed stomata'
1	16	3200	93	7
2	20	4000	30	3
3	24	2400	196	18
4	22	2200	22	5
5	28	3000	61	15
6	28	2800	300	14
7	—	264	27	6
8	—	660	23	1
9	—	200	17	4
Totals	138	18,724	769	73

were closed. The plants were inoculated and left in the moist chambers for four days more after which the leaves were fixed and counts made. Checks on the stomatal apertures were made occasionally throughout the period in the moist chamber. The stomata in the dark were almost all completely closed while most of those in the greenhouse remained open. It should be stressed here that neither in this experiment nor in any of the others reported was complete closure nor complete opening of all stomata observed. The counts showed that 28 penetrations occurred in the dark and 319 in the light.

In Experiment 4, leaves were treated as in the above case except that one lot of leaves was prestarved in the dark prior to inoculation. Both lots were inoculated and placed in dark, moist chambers. The prestarved plants showed closed stomata throughout the infection period, while the others remained open for 48 hr. after inoculation. At the end of that period they were fixed and examined. Five penetrations occurred in the leaves with closed and 22 in those with open stomata. Unfortunately this experiment could not be repeated within the season of light intensity that was essential to the stomata of unstarved plants remaining open for any considerable period in the dark.

In Experiments 5 and 6, leaves on cut stems were inoculated and placed in moist chambers in the greenhouse over sulphuric acid to give a relative humidity of 95%. Half of the stems were placed in a flask with water and half in an empty flask. Counts made on the third day after inoculation showed that 29 penetrations had occurred into the leaves with closed stomata, and 361 into those with open.

Experiments 7, 8, and 9 were carried out on strippings on sucrose solutions. Leaves were inoculated and placed in moist chambers until some germination had occurred. They were then stripped and floated on solutions in covered watch glasses. After two days the strippings were mounted in lactophenol cotton blue. Eleven penetrations occurred through closed and 67 through open stomata.

Throughout the nine series, 73 penetrations were observed into the leaves with closed and 769 into those with open stomata. Results with all methods were consistent in indicating many more penetrations where the stomata were open. It must be admitted that at least one factor varies in all the above experiments, but it is felt that the number of methods used and the consistency of the results answers any criticism based on these differences. We may conclude that penetration of sweet pea leaves by *C. album* is greatly impeded by closure of the stomata.

It is interesting to compare these results obtained with *C. album* with those of other workers. Hart (13) evolved a theory of "functional resistance" of wheat to stem rust based on the inability of the rust to penetrate closed stomata, but this "functional resistance" was questioned by Peterson (21). Later, Caldwell and Stone (3) showed that germ tubes of *Puccinia triticina* Eriks. penetrated closed stomata as frequently as open. Hart and Forbes (14) investigated a number of rusts and found that light experience at the time of infection influenced the amount of infection in some cases but did not in others. Pool and McKay (22) reported that germ tubes of *Cercospora beticola* Sacc. did not penetrate closed stomata of the sugar beet.

With the exception of Pool and McKay (22) all the reports mentioned above deal with the rusts. Even in that group the behaviour does not appear to be consistent, though it seems that at least some rusts penetrate closed stomata. Possibly they are able to do this by virtue of the appressorium, which usually forms over the stoma. So far as is known, penetration through closed stomata has not been shown for fungi that do not form appressoria such as *C. album* and *Cercospora beticola*. This suggests that exclusion may be mechanical, though evidence will be presented in the following section that a type of exclusion may result from suppression of factors that guide the germ tubes to the stomata. It may be that there is a fundamental difference in the mechanisms of penetration with and without appressoria, but further comparative work is necessary before any conclusion can be reached on this point.

The Role of Hydrotropism in Penetration

Hydrotropism on an Artificial Membrane

It has been suggested a number of times (Bond (2), Balls (1)) that germ tubes of fungi that normally penetrate the host through stomata are attracted to these by the water vapour transpired. As a preliminary test of this hypothesis an experiment was designed to see whether *C. album* showed any hydrotropism under controlled conditions that simulated those of the leaf epidermis. The data obtained in the experiments on *C. album* have been augmented by several series on *C. fulvum*.

Experiments were set up in the following manner. Van Tieghem cells were cemented to microscope slides, and the upper rim covered with a layer of paraffin. The cell was half-filled with water, and a piece of very thin aluminium foil was sealed over the cell by being pressed down around the edges with

a warm spatula. The foil was pierced with small holes about 50μ in diameter and 2 mm. apart using a fine glass needle and spores from a young colony of *C. album* or *C. fulvum* dusted over the surface of the membrane. This was done by lifting the surface of an agar slant culture with a long spatula. The agar stuck to the spatula sufficiently that the culture could be inverted over the membrane and pressed lightly on it, dislodging a large number of spores. The slides were then placed in moist chambers in which the humidity was controlled. In most series a humidity of 95% was obtained with a sucrose solution, or 100% with pure water. The moist chambers were placed in a constant temperature oven at 25°C .

Observations were made on the slides after two or three days depending upon the rapidity with which the spores germinated. The method of studying the effect of the conditions imposed was adapted from that of Graves (9) in which the directions of large numbers of germ tubes growing adjacent to the holes was analysed to show whether or not there was any directive force. Only spores lying one diameter of the hole or less from its edge were considered. Imaginary crossed lines giving quadrants, *A*, *B*, *C*, and *D*, were placed upon each spore and the germ tubes classified according to the quarter into which they grew. A preponderance of *A* types would be expected if there were an attraction towards the hole, a preponderance of *C*, if there were a repulsion, and equal numbers of all if there were no effect.

The results of the experiments are given in Table II. In all cases in which there was a gradient of increasing moisture as the holes were neared, there was a considerably larger number of germ tubes growing towards the hole than away from it. In the control experiments where there was no moisture gradient, there was no significant difference in the numbers in the various classes.

TABLE II

DIRECTION OF GROWTH OF GERM TUBES ON A PERFORATED ALUMINIUM MEMBRANE SEPARATING AREAS OF DIFFERENT RELATIVE HUMIDITIES. CLASS *A* INCLUDES THE GERM TUBES GROWING TOWARDS THE HOLE, CLASS *C*, THOSE AWAY FROM THE HOLE, AND CLASSES *B* AND *D*, Laterally

<i>R.H.</i> (ext.)	<i>R.H.</i> (int.)	Fungus	Class <i>A</i>	Class <i>B</i>	Class <i>C</i>	Class <i>D</i>
95	100	<i>C. album</i>	48	20	15	24
95	100	"	38	9	6	9
95	100	"	24	7	1	11
95	100	<i>C. fulvum</i>	21	2	0	1
95	100	"	31	1	2	2
95	100	"	113	27	14	26
95	100	"	173	56	53	59
95	100	"	448	194	105	201
100	100	<i>C. album</i>	8	7	7	7
100	100	<i>C. fulvum</i>	86	79	87	74
100	100	"	45	41	47	39
100	100	"	96	91	89	90

The behaviour of germinating spores on an aluminium membrane under the conditions imposed shows that there is a hydrotropic response in both *C. album* and *C. fulvum* to a gradient of the magnitude likely to be found on a leaf during the inoculation period. This demonstration of the hydrotropic response of *C. album* and *C. fulvum* is one of the few dealing with the response of fungi to volatile substances. Balls (1) noted that germ tubes of *Puccinia glumarum* var. *Hordei* Eriks. grew from room air through perforations in a rubber membrane towards a saturated atmosphere but showed no tendency to return. Miyoshi (19), Fulton (7), and Graves (9) have dealt with chemotropism of fungi in, or on, a liquid or agar substrate, but their results have little or no bearing on problems of stomatal penetration. It is hoped that the methods described here may prove applicable to a study of the influence of volatile substances other than water on the direction of growth of germ tubes.

Hydrotropism on the Leaf

If it be true that germ tubes are attracted to stomata when the air at their mouths is more humid than in the regions of the germ tube, then the number of penetrations should be increased by an increase in the circumstomatal gradient. This increase may be achieved by decreasing the relative humidity of the air around the leaf. The influence of the gradient on the frequency of penetration should then be shown by an increase in the number of penetrations with decreasing humidity. Humidity values given are those at the surface of the control solutions approximately one-half inch away from the leaf.

It was not found possible to make a direct measurement of the effect of relative humidity on the frequency of penetration. First experiments in which inoculated leaves were incubated in moist chambers over sucrose solution to give relative humidities of 90 to 100% (Shaw (23)) showed that the number of penetrations tended to increase with a decrease from 100% to 97% of saturation and then decreased till there were very few at relative humidity 90%. There was evidence in these experiments that the sharp reduction in growth in the lower 90's was responsible for the decrease in number of penetrations.

Since it appeared that the real influence of humidity on frequency of penetration could only be determined after eliminating the variations due to different extents of growth, an investigation of the relation between number of penetrations and length of germ tubes was carried out. Leaves were inoculated, placed in control chamber at relative humidity 100%, and then in constant temperature growth chambers at 22.5° C. and 15° C. (12° in one experiment). At intervals of approximately 12 hr., samples of four leaflets were removed and the frequency of penetration and average length of germ tubes estimated. Results of three such pairs of experiments are given in Table III. Judging by the correlation coefficients, there is a close approximation to a linear relation between length of germ tube and frequency of penetration. This provides us with a means of obtaining comparable frequencies even when the extent of growth is not equal.

TABLE III

RELATION OF AVERAGE LENGTH OF GERM TUBES IN μ TO NUMBERS OF PENETRATIONS

Series No.	Temperature, ° C.	Paired readings					Correlation coefficient	Regression coefficient	
1	22.5	Length	0	62	122	162	205	.99**	2.4 ± .2
		Penetration	0	20	38	67	94		
	15	Length	0	37	91	178		.91*	2.1 ± .6
		Penetration	0	10	34	68			
2	22.5	Length	0	56	125	105	195	.98**	5.3 ± .7
		Penetration	0	51	109	190	216		
	15	Length	0	86	101	151	183	.98**	5.3 ± .6
		Penetration	0	85	121	155	175		
3	22.5	Length	0	45	97	173	226	.98**	2.9 ± .3
		Penetration	0	14	27	83	119		
	15	Length	0	52	70	92	124	.92*	2.0 ± .5
		Penetration	0	19	30	47	39		

** Correlation coefficients significant beyond the 1% level.

* Correlation coefficients significant beyond the 5% level.

With this information available, further experiments were conducted to determine the influence of relative humidity independent of its effect on growth. Inoculated leaves were placed over control solutions to give relative humidities of 90, 94, 96, 98, and 100%, and the control chambers maintained at 22.5° C. and illuminated for 12 hr. each day. In order to allow for approximately the same amount of growth at all humidities, leaves were fixed after various periods in the control chamber. About 40 hr. was allowed for those at relative humidity 100% and up to 80 hr. for those at 90%. The extent of growth was estimated by measuring the lengths of 100 germ tubes and taking the mean. The counts of penetrations and average lengths of germ tubes, together with the 'corrected' values for the counts are given in Table IV, the 'corrected' values being obtained by multiplying the observed number of penetrations by the factor 'average length of germ tube at relative humidity 100%/average length of germ tube at humidity concerned'. The results of the three experiments given in Table IV are shown graphically in Fig. 11. There is a striking increase in the frequency of penetration as the humidity decreases providing the same degree of growth is permitted. Over six times as many penetrations occurred per unit of growth at relative humidity 90% as at relative humidity 100%.

It may perhaps be suggested that the germ tubes that grow slowly may respond more to directive forces on the leaf than those that grow more rapidly, and that this may explain the results obtained. However, if the rate of growth had such an effect, the frequency of penetration at low temperatures where growth is reduced should be greater than that at higher, and the value

TABLE IV
INFLUENCE OF HUMIDITY ON FREQUENCY OF PENETRATION

Relative humidity	No. of penetrations in 200 fields				Average length of 100 germ tubes in μ				Penetration corrected for extent of growth			
	Expt.				Expt.				Expt.			
	1	2	3	4	1	2	3	4	1	2	3	4
100	79	76	348	98	70	97	140	160	79	76	348	98
98	172	170	489	154	70	86	160	115	172	192	392	221
96	297	235	870	146	65	81	160	92	319	282	690	257
94	259	363	1116	142	54	65	145	—	336	544	1070	—
90	624	207	1277	14	65	41	102	—	676	495	1750	—

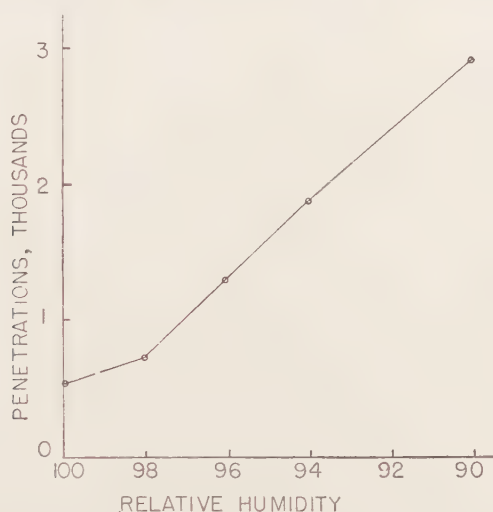


FIG. 11. The effect of relative humidity on frequency of penetration. The graph is a summary of Experiments 1, 2, and 3 of Table V and each point represents the 'corrected' value for number of penetrations in 3600 fields on 18 leaflets.

for the regression coefficients for growth and penetrations (Table III) at 15° should be greater than those at 22.5° . This is not the case. The effect of temperature in these series might possibly be such that a tendency for decreasing rate of growth to increase the frequency of penetration was exactly counteracted, but this is extremely unlikely. We may therefore conclude that the rate of growth is not a factor and that the results of the humidity experiments of Table IV show the influence of relative humidity on frequency of penetration, independent of any effect due to rate of growth.

In addition to these experiments on the effect of constant humidity on frequency of penetration, several were done in which the number of penetrations occurring in constant saturation and in fluctuating humidity were compared. These were modelled somewhat after Bond's (2) on *Cladosporium*

fulvum and consisted of placing inoculated leaves in large jar moist chambers in the greenhouse. Half of the leaves were maintained in a saturated atmosphere throughout the experiment, and the other half in a saturated atmosphere during the night and in one of 90% relative humidity during the day. Results of three such experiments are given in Table V.

It was found that over three times as many penetrations occurred in fluctuating humidity as in constant saturation. These results agree with Bond's and support the hypothesis that germ tubes enter in greater numbers when there is a water vapour gradient around the stomata.

TABLE V
FREQUENCY OF PENETRATION OF *C. album* IN ATMOSPHERE OF
CONSTANT SATURATION AND FLUCTUATING HUMIDITY

Experiment	Number of penetrations in constant saturation	Number of penetrations in fluctuating humidity
1	79	319
2	14	52
3	107	300

The results of all the humidity studies are consistent in supporting the theory that water vapour attracts the germ tubes to the stomata. It may be felt that the differences of relative humidity are too small to make a definite reaction probable. However, Clayton (4) has shown that under carefully controlled conditions many fungus spores are extremely sensitive to small changes in humidity. This would support the contention that fungi may react to a small gradient around the stomata.

Acceptance of the importance of hydrotropism in infection does not preclude the possibility that other factors may also exert a directive influence on the germ tube. If the direction of growth of the hyphae on the leaf were controlled entirely by gradients of water vapour, then no penetrations should occur at relative humidity 100%, since the air in the leaf must be slightly less than saturated. Experiments reported under the study of stomata showed that leaves whose petioles were not supplied with water became somewhat flaccid even in a 'saturated' atmosphere and it may be that the actual value of the relative humidity of the air over pure water in these experiments sometimes dropped enough to give a gradient around the leaf. It is also possible that the penetrations that occurred were purely chance. On the other hand, there may be other substances diffusing from the stomata to which the fungus is positively tropic and this seems the more probable explanation. It is concluded that germ tubes of *C. album* are attracted to the stomata of sweet pea by a positive water vapour gradient and may be attracted by other factors. There is evidence presented above, and in Bond's (2) work, that this also applies to *C. fulvum*.

Host Range

A number of leguminous plants was tested for susceptibility to *C. album*. This was done by inoculating sweet peas and the species to be tested at the same time and placing the plants in a moist chamber until the sweet peas showed distinct symptoms of disease. All were then examined and areas that showed any indication of disease were cleared in lactophenol and examined under the microscope. Each species was tested on at least two occasions. The following are the species that were tested:

Lathyrus latifolius L.

Vicia faba L.

Vicia villosa Roth.

Melilotus alba Desr.

Medicago sativa L.

Pisum sativum L.

Phaseolus vulgaris L.

Trifolium pratense L.

Glycine Max Merr.

Penetrations were observed in a number of these species, viz. *L. latifolius*, *M. sativa*, *P. sativum*, *T. pratense*, and no doubt occurred in others.

The relative frequency of penetration into pea and sweet pea was estimated by placing inoculated leaves over a sugar solution to give relative humidity 94% and by counting penetrations in 200 fields per leaflet. The results showed 277 penetrations on eight leaflets of pea and 365 on eight leaflets of sweet pea. More penetrations occurred on sweet pea but its rougher leaves appeared to retain more spores, so that the difference cannot be considered significant. Apparently *C. album* may penetrate leaves that it does not infect with approximately the same frequency as those that it does. Such behaviour on the part of parasitic fungi has long been known for the rusts (Gibson (8), Hanes (10)), and has also been shown for certain cases of direct penetration (Johnson (16)). These studies on *C. album* again emphasize that penetration is not a function of a particular host-parasite reaction. While they do not in themselves support the idea that hydrotropism is an important factor in penetration, they indicate that some such non-specific factor or factors are of major importance.

In none of the plants tested did the fungus develop sufficiently to be considered as pathogenic although, on one occasion, appreciable development took place in red clover and, on another occasion, several places were found where the fungus had spread rather widely through a leaf of *P. sativum*. Attempts to duplicate these results were unsuccessful. The lesions were barely visible, and were shown to be caused by the fungus only after microscopic examination. Isolation was therefore impossible. It may be that a race having some potentialities to parasitize *P. sativum* was responsible for these cases, though Dowson found that neither *P. sativum* nor *Lathyrus aphaca* was attacked by the culture he studied. However, Hansford (11) reports *C. album* on bean and pea and again (12) on bean and vetch in Uganda, and Pape and Englehardt (20) report an attack on greenhouse vetches in Germany. It appears that within the species *C. album* there may be races pathogenic to leguminous plants other than the sweet pea.

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STUDIES ON *FUSARIUM CULMORUM* BLIGHT OF CRESTED WHEAT AND BROME GRASS SEEDLINGS¹

BY JOHN T. SLYKHUIS²

Abstract

A number of pathogenic fungi were isolated from blighted brome grass and crested wheat grass seedlings grown in Saskatchewan and Ontario soils. The parasitism of one of the widely distributed and commonly occurring of these, *Fusarium culmorum* (W. G. Sm.) Sacc., was studied in more detail.

One per cent of *F. culmorum* sand-cornmeal inoculum caused more blight of brome grass seedlings in sterilized soil than did 6% in unsterilized soil. The development of *F. culmorum* in sterilized soil was optimum at 25° C. and declined rapidly with rising and more slowly with falling temperatures. Seedling blight was severe in sterilized soil at all temperatures from 10° to 35° C., but was significantly more severe near the optimum for the fungus provided the soil was not infested too heavily. In unsterilized soil, however, both the development of *F. culmorum* and the incidence of seedling blight were much greater at 10° to 20° C. than at 25° C. and higher, whereas other soil fungi and bacteria were more numerous at 25° C. and above than at the lower temperatures.

An infusion of unsterilized soil, a suspension of miscellaneous soil bacteria, and a mixture of 75 soil fungi suppressed the development of *F. culmorum* in sterilized soil, and also caused reductions in seedling blight. Of 136 soil fungi tested, only three reduced fusarial blight in sterilized soil. These antagonistic fungi included isolates of *Acremonium*, *Gliocladium fimbriatum* Gilman and Abbott, and *Phialophora*. Their ability to reduce disease incidence was not consistently correlated with the production of toxic filtrates, or the inhibition of *F. culmorum* in culture or in the soil but it was related to the effect they had on the development of *F. culmorum* in the environment in the immediate vicinity of the germinating seeds. This zone within which the germinating seed induces a characteristic change in the microbiological balance is designated as the 'spermatosphere'.

Disease incidence varied among different unsterilized field soils uniformly infested with *F. culmorum* and in these experiments was more severe in clay than in the soils of lighter texture. There was no consistent correlation between the suppression of blight and the numbers of fungi, bacteria, or actinomycetes in the different soils, but there was a correlation with the numbers of bacteria in the spermatosphere.

Preliminary Survey of Grass Parasites

In view of the increasing importance of forage grasses in prairie agriculture, the diseases that may reduce their stand, yield, or longevity, or have an effect on other crops in the same rotation, assume a new significance. Parasites of the seedling stage are of such potential significance that studies were undertaken to gain further information on the diseases of grass seedlings and on certain factors influencing the activity of the associated pathogens that are harboured in the soil. Since crested wheat grass (*Agropyron cristatum* (L.) Gaertn.) and brome grass (*Bromus inermis* Leyss.) are the most important of the cultivated forage grasses in the Canadian prairies at the present time, these two were selected as hosts in the experimental work.

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When these grasses were sown in soil obtained from fields in various districts of central Saskatchewan, emergence was generally lower than in sterilized soil, and varying percentages of the seedlings that emerged died later. To discover the cause of this, fungi were isolated from well-washed seedlings that had been killed or damaged at various stages of development. The pathogenic fungi thus obtained included isolates of *Fusarium arthrosporioides* Sherb., *F. culmorum* (W. G. Smith) Sacc., *F. Equiseti* (Corda) Sacc., *F. oxysporum* Schlecht, *F. Scirpi* var. *acuminatum* (Ell. and Ev.) Wollenw., *F. Solani* (Martius) Appel. and Wollenw.,* *Helminthosporium sativum* P.K. and B., *Pythium debaryanum* Hesse, *P. ultimum* Trow., and two isolates whose identity was not determined because they did not sporulate. All of the above-mentioned pathogens reduced emergence. *Pythium* species caused a certain amount of damping-off while some of the *Fusarium* isolates caused wilting and death, and often a brownish discoloration of the stem bases of seedlings. *H. sativum*, in addition to causing wilting and death of emerged seedlings, produced dark brown to black lesions on the stem of the seedling that resulted in stunting if not in killing the young plant. In addition this fungus occasionally caused dark lesions on the leaves, but the plants survived despite this type of injury. A comparison of the stands of crested wheat grass seedlings two weeks after sowing in sterilized soil infested with a number of pathogenic isolates is given in Table I.

TABLE I

THE INFLUENCE OF PATHOGENIC FUNGI ON THE STAND OF CRESTED WHEAT GRASS SEEDLINGS IN STERILIZED SOIL

Inoculum	Number of isolates tested	Average stand, %
Check	—	91
<i>Fusarium arthrosporioides</i>	1	58
<i>F. culmorum</i>	5	9
<i>F. Equiseti</i>	3	70
<i>F. oxysporum</i>	1	72
<i>F. Scirpi</i>	2	7
<i>F. Solani</i>	1	50
<i>Helminthosporium sativum</i>	16	12
<i>Pythium debaryanum</i>	4	56
<i>P. ultimum</i>	2	71
Unidentified isolate	1	64

* The species of *Fusarium* were identified by Dr. L. W. Gordon, Dominion Laboratory of Plant Pathology, Winnipeg, Man.

Pre-emergence blight caused by these pathogenic fungi was about equally severe in crested wheat grass and brome grass, but under the conditions of these experiments postemergence blight was more severe in crested wheat grass than in brome grass. Later experiments showed that susceptibility to the latter type of blight was influenced by temperature. Both grasses were more susceptible at temperatures of 25° C. and higher than at the lower

temperatures, and although there was little difference in susceptibility of the two grasses at the higher temperatures, brome grass was less susceptible than crested wheat grass at moderate and low temperatures.

Isolations from crested wheat and brome grass seeds yielded a large number of various fungi, among which were pathogenic isolates of *F. culmorum*, *F. Equiseti*, *F. Scirpi* var. *acuminatum*, and *H. sativum*. Further isolations were made from 39 samples of brome grass and 35 of crested wheat grass seeds grown in widely scattered areas of Saskatchewan and Manitoba in 1942. *H. sativum* was detected in only two samples of brome and five of crested wheat grass seeds. *Fusarium* isolates were obtained from two samples of brome and 21 of crested wheat grass seeds but not all of these isolates were pathogenic.

The Parasitism of *Fusarium culmorum*

It was the purpose of the experiments that follow to study some of the factors that influence seedling blight caused by *F. culmorum*, one of the more important and widely distributed pathogens of cereal and forage grass seedlings, especially on the Canadian prairies. These studies have dealt chiefly with the influence of temperature and of soil microflora on disease incidence.

A. The Survival and Virulence of *Fusarium culmorum* in Sterilized and Unsterilized Soil

The virulence of *F. culmorum* was compared in sterilized and unsterilized Saskatchewan black loam soil. Inoculum was prepared by growing *F. culmorum* for one month in flasks containing a sterilized moist mixture of sand and 2% cornmeal by weight. This inoculum was mixed with quantities

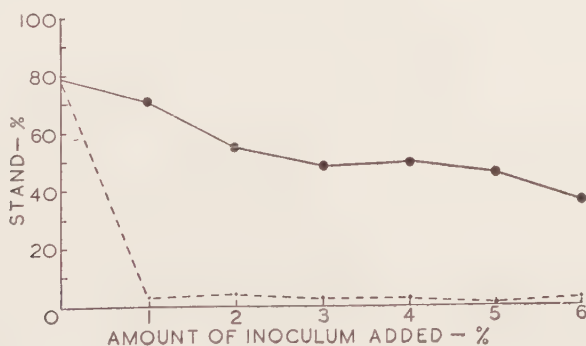


FIG. 1. Influence of the amount of *F. culmorum* inoculum on seedling blight of brome grass in sterilized and unsterilized portions of a Saskatchewan black loam soil. ----- Sterilized. ————— Unsterilized.

of each soil at the rates of 0, 1, 2, 3, 4, 5, and 6% by weight, and two days later 50 brome grass seeds were sown in two 6 in. pots of each treatment. The percentage stand of seedlings two weeks after sowing is plotted in the graph in Fig. 1 against the percentage of inoculum in the test soils. The stands.

were about equal in the two soils in the absence of inoculum. When the sterilized soil was infested with *F. culmorum* the stand was drastically reduced, and 1% of inoculum reduced seedling survival as effectively as larger amounts. In the unsterilized soil, on the other hand, 1% of inoculum reduced the stand only slightly, and greater concentration of inoculum caused greater reductions in stand, but even 6% of inoculum in unsterilized soil reduced the stand less than half as much as did 1% in sterilized soil. This suggests that biotic factors are exerting a strong influence in unsterilized soil.

Further evidence of the importance of the biotic factors in the soil was supplied by other experiments, which showed that seedling blight was reduced by the addition of well-rotted manure to sterilized and unsterilized soil infested with *F. culmorum* inoculum (13). Also, when sterilized and unsterilized soils were re-sown five months after infestation with *F. culmorum*, there was no seedling blight caused by this fungus. Evidently under the conditions of the experiment the *F. culmorum* added with the inoculum was unable to establish itself at the high concentration among the normal soil flora, and was crowded out by the micro-organisms that repopulated the sterilized soil.

In another experiment the stands of brome grass seedlings in freshly sterilized and unsterilized soil infested with 6% *F. culmorum* inoculum were compared with the stand in similarly infested pots of soil that had been sterilized one year earlier and allowed to stand in the greenhouse. The results (Table II) showed that as usual the stands in the freshly sterilized soil were much lower than in the unsterilized soil equally infested with *F. culmorum*.

TABLE II

THE RELATION BETWEEN MICRO-ORGANISM POPULATIONS AND SEEDLING STANDS
IN UNSTERILIZED AND REPOPULATED STERILIZED SOILS
UNIFORMLY INFESTED WITH *F. culmorum*

Soil	Stand, %	Fungi/gm.	Bacteria/gm.
Freshly sterilized	6		
Sterilized 1 year previously	46	1,650,000	12,670,000
Unsterilized	36	100,000	4,810,000

In the soil that had been sterilized one year earlier, however, the stand was 10% higher than in the unsterilized soil. The numbers of bacteria and fungi populating the old sterilized and unsterilized soils were investigated by the dilution plate method, using peptone-glucose acid agar for the fungi and sodium albuminate agar for bacteria (3). The counts showed that the bacteria were about three times and the fungi about 16 times more abundant in the old sterilized than in the unsterilized soil. This suggests that the suppression of seedling blight caused by *F. culmorum* may be a function of the number of micro-organisms populating the soil.

The survival of *F. culmorum* in sterilized soil watered with sterile water and with an infusion of unsterilized soil was investigated. Three textures of soil, clay loam, loam, and sandy loam were used. One hundred and fifty grams of each type of soil was put into each of two 300 cc. Erlenmeyer flasks, which were then plugged with cotton and sterilized for two hours at 15 lb. pressure. Two days after sterilization each flask was inoculated with 10 cc. of a suspension of *F. culmorum* spores in sterile water. To one flask of each type of soil 10 cc. of sterile water was added, and to the other 10 cc. of an infusion made by shaking 5 gm. of a Saskatchewan loam soil with 100 cc. of sterile water, then allowing the heavy soil particles to settle, and pouring off the liquid. The flasks were left undisturbed for 24 hr., then shaken vigorously to mix the soil thoroughly. At this time counts were made by the dilution plate method of the numbers of *F. culmorum* isolates per gram. The flasks were kept at room temperature and further counts were made 7, 23, and 55 days after inoculation. Since the numbers of isolates from the different soils similarly treated were not very different, the results given in Table III are the averages of the counts from the three soils.

TABLE III
THE INFLUENCE OF AN INFUSION OF UNSTERILIZED SOIL ON
THE DEVELOPMENT OF *F. culmorum* IN STERILIZED SOIL

Days after inoculation	<i>F. culmorum</i> isolates per gram after the addition of:	
	Sterile water	Soil infusion
1	11,600	7900
7	94,100	36,600
23	124,300	39,300
55	47,200	13,000

In both series of flasks the numbers of isolates were highest in the counts made on the 23rd day after inoculation, and were greatly reduced by the 55th day. The latter reduction may have been accentuated by the slow drying out of the soil. The most significant feature of the results, however, was that on and after the seventh day the numbers of *F. culmorum* isolates were much higher in the soils to which sterile water had been added than in those moistened with the infusion of unsterilized soil. Colonies of soil fungi were frequent on the medium on which dilutions of the latter soils were plated for counting *F. culmorum* isolates, and when dilutions were plated on sodium albuminate agar it was found that on and after the seventh day there were more than twenty million bacteria per gram. Evidently the development of fungi and bacteria, which were added with the unsterilized soil infusion, suppressed the development of *F. culmorum*.

Experiments with suspensions of miscellaneous soil bacteria and with mixtures of 75 miscellaneous soil fungi (13) have shown that when added to

sterilized soil infested with *F. culmorum*, both bacteria and fungi retard the development of the pathogen in the soil and reduce the incidence of seedling blight. It is therefore likely that the suppression of seedling blight in artificially infested unsterilized soil is a result of the suppression of the development of *F. culmorum* by the micro-organisms inhabiting the soil.

B. The Relation of Temperature to the Development and Pathogenicity of *Fusarium culmorum*

1. In Sterilized Soil

The influence of temperature on the development of *F. culmorum* in sterilized soil was investigated. One-hundred-gram quantities of black loam soil were autoclaved in 250 cc. Erlenmeyer flasks. To each, 15 cc. of a heavy suspension of *F. culmorum* spores was added, then these flasks of inoculated soil were incubated at six temperatures ranging from 10° to 35° C. Special precautions were taken to reduce evaporation and the flasks were shaken periodically to mix the soil thoroughly. After 12 days' incubation the numbers of *F. culmorum* isolates per gram of soil were counted by the dilution plate method. In another experiment the influence of temperature on the radial growth of colonies growing on an agar medium was investigated throughout a range of 5° to 40° C. The results of these experiments are presented in Fig. 2 from which it is apparent that the graphs obtained by the two methods were very similar. In both experiments development was greatest at 25° C., but with lowering temperatures it decreased gradually, while with rising temperatures it decreased more rapidly.

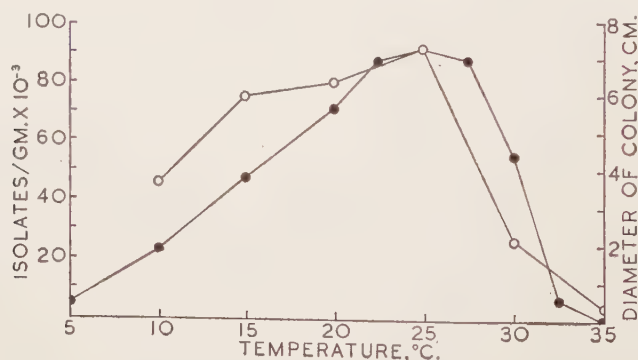


FIG. 2. Influence of temperature on *F. culmorum* development on agar and in soil. ● = Radial growth after three days on potato dextrose agar. ○ = Isolates per gram of soil.

Wisconsin temperature tanks were used to study the influence of soil temperatures on the incidence of blight in brome and crested wheat grass seedlings. The six tanks, each of which was fitted with eight metal cans 18 cm. in diameter and 30 cm. deep, were adjusted to give soil temperatures ranging from 10° C. to 35° C. with 5° intervals. A steam sterilized mixture of three parts clay loam soil and one part sand was prepared. The soil for four cans in each tank was mixed in bulk with 1% of its weight in a

two week old culture of *F. culmorum* on a 2% cornmeal-sand mixture. The soil for the remaining four cans was not infested. Brome grass was sown in two cans of infested and two of uninfested soil in each tank, and crested wheat grass was sown in the others at the rate of 100 seeds per can. The moisture content of the soil was held near 40% of the moisture holding capacity. The development of seedlings was observed and the stands were counted three weeks after sowing in three replications of this experiment.

Both grasses began to emerge four days after sowing at 30° and 35° C., but at lower temperatures the time for emergence was increased and was eight or nine days at 10° C. Growth was slower but the seedlings were hardier at temperatures up to 20° C., while at higher temperatures growth was more rapid and the seedlings appeared chlorotic and spindly, and even in the uninfested soil some of them died, especially at 35° C. The influence of temperature on the stands of the two grasses was very similar. The results with brome are presented graphically in Fig. 3. In uninfested soil the stand

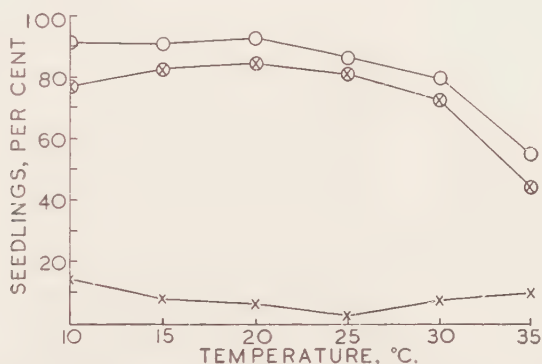


FIG. 3. Influence of temperature on blight and stand of brome grass seedlings in sterilized soil. O = Stand in uninfested soil. X = Stand in soil infested with *F. culmorum*. ⊗ = Blight caused by *F. culmorum*.

was uniformly high at temperatures up to 25° C., but at higher temperatures than this it was significantly lower. Thus, although high soil temperatures increased the rate of emergence and growth, low temperatures were more favourable for high stands of vigorous seedlings. In soil infested with *F. culmorum* the stand was low at all temperatures but was lowest at 25° C.

The amount of seedling blight caused by *F. culmorum* is indicated by the differences between the stands in infested and uninfested soils, and was greatest at temperatures of 15° to 25°C., the temperature range most favourable for the development of grass seedlings, and also for the development of *F. culmorum* in sterilized soil (Fig. 2). It appears, therefore, that in sterilized infested soil the incidence of seedling blight at different temperatures depends largely on the relative concentration of *F. culmorum* that develops in the soil. This was borne out in other temperature experiments in which higher rates of infestation of sterilized soil were used. With such higher rates of infestation

no seedlings survived except a small percentage at 30° and 35° C., hence the effect of temperature was largely obscured because of the high concentration of *F. culmorum* at all temperatures.

2. In Unsterilized Soil

In a preliminary experiment on the influence of temperature on blight of brome grass seedlings in unsterilized soil, a Saskatchewan black loam soil infested with various amounts of *F. culmorum* inoculum was used. The Wisconsin temperature tanks were employed to maintain six temperatures ranging from 10° to 35° C. Two cans in each tank contained soil infested with 4, 7, and 10% of inoculum while two others contained uninfested soil. One hundred brome grass seeds were sown in each can. The soils were kept moderately moist by frequent light sprinklings. The seedlings were counted 10 days after first emergence in each tank.

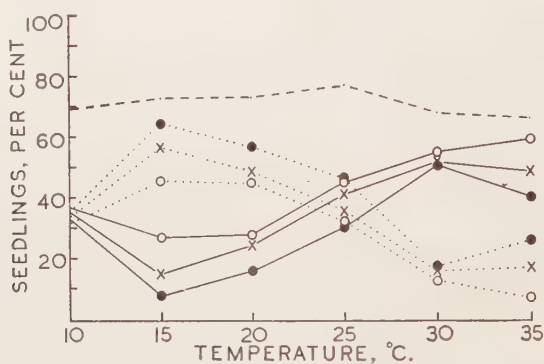


FIG. 4. The relation of temperature to disease incidence in unsterilized soil infested with different amounts of *F. culmorum* inoculum (○ = 4% inoculum, × = 7% inoculum, ● = 10% inoculum). — Stand in infested soil. Seedling blight caused by *F. culmorum*. ----- Stand in uninfested soil.

Fig. 4 shows the graphs of the stands of seedlings at different temperatures in the uninfested soil and in the soil infested at different rates with *F. culmorum*. In the uninfested soil the stand was slightly higher at 25° C. than at the higher and lower temperatures. In the infested soils, at every rate of infestation, the stands were lowest at 15° to 20° C., and highest at 25° to 35° C. The differences between the stands in the infested and uninfested soils indicate the amount of seedling blight caused by the *F. culmorum* added to the soil. At every temperature the severity of blight was related to the amount of inoculum that had been added to the soil, but the form of the graphs of the effect of temperature on blight was similar at every rate of infestation. In each instance blight was most severe at 15° C. but decreased with increasing temperatures to a minimum at 30° to 35° C. This is in striking contrast with results in sterilized soil where disease incidence was greatest at 25° C., which likewise is the optimum temperature for the development of the pathogen.

The influence of temperature on seedling blight and on the numbers of bacteria and fungi in two other Saskatchewan soils artificially infested with 4% *F. culmorum* was investigated in the same manner. They were black parkland soils, one a clay loam, the other a sandy loam, and both had grown cereal crops for a number of years.

Although the stands were consistently lower in the infested clay loam than in the sandy loam soil, the graphs of the influence of temperature on stand were similar in form for both. The average of the results from the two soils is presented in Fig. 5. In the uninfested checks the stand was slightly higher at 25° C. than at either higher or lower temperatures. In the infested soil the stand was lowest at 15° C. and increased with rising temperatures to a maximum at 35° C. The incidence of seedling blight thus was greater at 15° C. than at the higher temperatures.

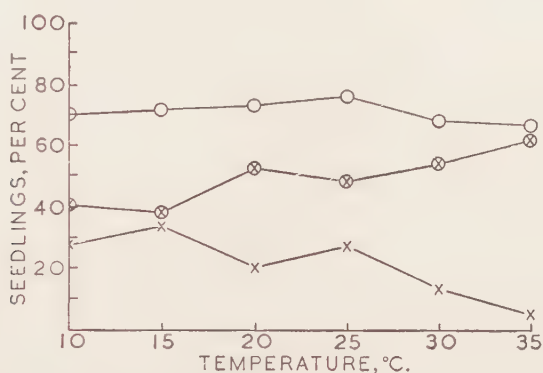


FIG. 5. The influence of temperature on percentage stand and seedling blight of brome grass in Saskatchewan field soil not infested and infested with *F. culmorum*. O = Stand in uninfested soil. X = Stand in infested soil. ⊗ = Seedling blight in infested soil.

The numbers of micro-organisms in the above two soils were counted after they had been kept in the tanks at the six temperatures for 90 days after infestation. The counts were made from dilution plates using sodium albuminate agar for the bacteria and peptone glucose agar for the fungi. Other fusaria were negligible in the soils and since *F. culmorum* colonies could be distinguished from those of other fungi, all were counted on the same plates. The counts of these micro-organisms for the two soils were averaged and the graphs of the results are shown in Fig. 6. *F. culmorum* isolates were most abundant at 15° C. and slightly less at 10° C. The numbers decreased with increasing temperatures to a minimum at 35° C. There was no marked influence of temperature on the numbers of other fungi in the soil. The bacteria, however, were significantly more numerous in the soils at 20° C. to 35° C. than at 10° and 15° C. Hence, in these soils, three months after being infested with *F. culmorum*, bacterial numbers were greater at the higher temperatures while the numbers of *F. culmorum* isolates were higher at the lower temperatures where seedling blight was most severe.

The influence of temperature on seedling blight and the numbers of micro-organisms in infested Ontario soils was likewise investigated. Four soils collected near Vineland, Ont. were selected to represent different textures and

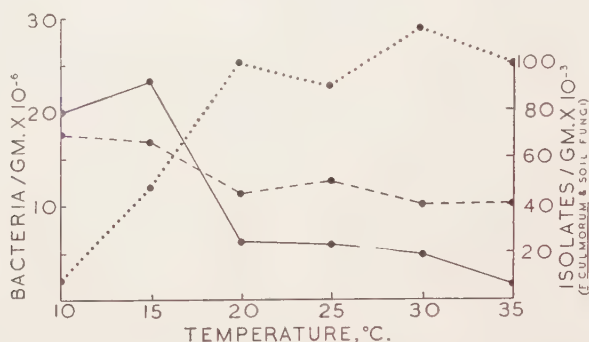


FIG. 6. The populations of bacteria, fungi, and *F. culmorum* in Saskatchewan field soil. (*F. culmorum* inoculum was added to the soil three months previously and the soils were kept at different temperatures during that time.) ——— *F. culmorum*. ---- Fungi. Bacteria.

cultural histories. One was clay, the other three were loam. The clay and one loam were from a field growing an alfalfa-timothy mixture. Another loam was from an oat field, and the third was from grass sod. The soils were infested with 4% *F. culmorum* inoculum, and two cans of each were included in each temperature tank and 100 brome grass seeds were sown per can. The experiment was repeated and the results averaged. Although blight was consistently more severe in the clay than in the other soils, the forms of the graphs of the influence of temperature on blight were similar for all, hence the results were averaged and the graph so obtained is presented in Fig. 7. At the conclusion of the second experiment to determine seedling

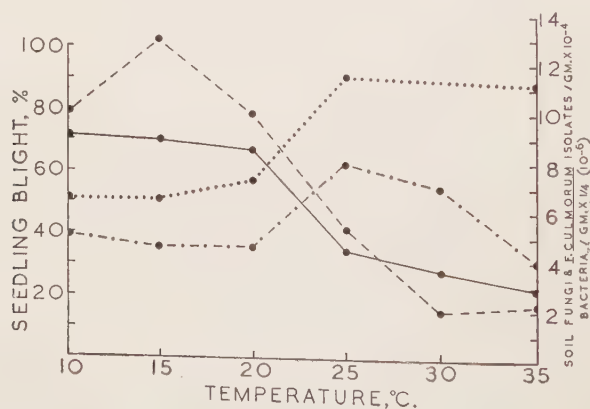


FIG. 7. The effect of soil temperature on numbers of *F. culmorum* isolates, other fungi, and bacteria, and on blight of brome grass seedlings in Ontario soil infested with 4% *F. culmorum* inoculum. ---- *F. culmorum*. Soil fungi. Bacteria. ——— Blight.

blight, which was 40 days after infestation, counts were made of the numbers of micro-organisms in the soils. The results from the four soils were averaged, and the resulting graphs of the numbers of *F. culmorum* isolates, fungi, and bacteria are presented, with the graph of seedling blight, in Fig. 7.

In these experiments with infested Ontario soils, as was the case also with Saskatchewan soils, seedling blight was more severe at the lower (10° to 20° C.) than at the higher temperatures (25° to 35° C.). Similarly the development of *F. culmorum* was greater at the lower than at the higher temperatures while bacteria were more abundant at the higher temperatures. In this experiment with Ontario soils the fungi too were somewhat more abundant at 25° to 30° C. than at lower temperatures.

These experiments on the influence of temperature on the incidence of seedling blight and the development of *F. culmorum* have revealed sharp contrasts between sterilized and unsterilized soils. In sterilized soil both disease incidence and the development of the pathogen were optimum at 25° C., but in unsterilized soils they were greater at much lower temperatures. On the other hand the development of bacteria, and in some instances of soil fungi, was greatest at and above the temperature that is optimum for *F. culmorum* development in sterilized soil. These results indicate that the incidence of blight is dependent on the development of *F. culmorum* in the soil, which in turn is greatly influenced by soil micro-organisms.

C. *The Antagonistic Effects of Certain Soil Fungi to F. culmorum*

From a large number of fungi isolated from an Ontario loam soil and from a Saskatchewan loam from a field that had grown cereals for a number of years tests were made for antagonism to *F. culmorum* with 110 isolates from the Saskatchewan and 26 from the Ontario soil. The influence of isolates of the soil fungi on the pathogenicity of *F. culmorum* was tested in sterilized soil infested with 2% of *F. culmorum* soil – oat hull inoculum. Enough of this soil for two 5 in. pots was infested with 2% or more of the inoculum of an isolate to be tested, and two days later 25 grass seeds were sown in each pot. Antagonism to *F. culmorum* was indicated if an isolate increased the percentage of seedlings that survived.

The influence of these isolates on the growth of *F. culmorum* on an agar medium was also studied. A piece of agar cut from the edge of a growing culture of the isolate to be tested was placed 3 cm. from a similar piece of *F. culmorum* inoculum on potato dextrose agar in a Petri dish. After five to six days' incubation at 25° C. the association effects of the isolate and *F. culmorum* were observed for indications of antagonism.

On the basis of these preliminary tests, five isolates were selected for further studies. Three were selected because they increased the survival of grass seedlings in soil infested with *F. culmorum*. Two of these, *Gliocladium fimbriatum* Gilman and Abbott, and an *Acremonium* species, were from the Saskatchewan soil. The third, isolated from Ontario soil, belongs to the genus *Phialophora*. Isolates of *Trichoderma lignorum* (Tode) Harty and

Penicillium urticae Bain* were also selected because of their antagonism to *F. culmorum* on agar medium.

1. *The Influence of Selected Soil Fungi on the Incidence of Seedling Blight Caused by F. culmorum*

The influence of each of the five selected soil isolates, and of a mixture of 75 isolates including the latter five, on the stands of crested wheat grass and brome grass seedlings in sterilized soil uniformly infested and not infested with *F. culmorum* is shown in Table IV. For comparison the stands of brome

TABLE IV

THE INFLUENCE OF SOIL FUNGI ON THE STANDS OF CRESTED WHEAT AND BROME GRASS SEEDLINGS IN INFESTED SOIL

Inoculum added to sterilized soil	Stand, %			
	Crested wheat grass		Brome grass	
	Check	<i>F. culmorum</i>	Check	<i>F. culmorum</i>
Check	92	13	90	16
<i>Phialophora</i>	98	92	90	92
<i>Acremonium</i>	—	—	90	68
<i>Gliocladium</i>	82	76	88	68
<i>Penicillium</i>	92	14	86	12
<i>Trichoderma</i>	88	12	88	10
Mixture of 75 isolates	—	—	86	24
Unsterilized soil	—	—	84	68

grass seedlings in equally infested and uninfested unsterilized soil are also given. Inasmuch as none of the soil fungi was pathogenic, they caused no significant reduction in seedling stand in sterilized soil, whereas *F. culmorum* reduced the stand severely. The addition of *Phialophora* to soil infested with *F. culmorum* increased the stands of seedlings to equal that in uninfested soil. Similarly *Acremonium* and *Gliocladium* caused increases in stand. The increases caused by these fungi were less spectacular, but the stands in these instances were equal to the stand in unsterilized soil that was equally infested with *F. culmorum*. Neither *Penicillium* nor *Trichoderma* caused any increase in seedling survival in infested soil. A mixture of 75 isolates of soil fungi, including the above five, caused only a slight increase in seedling survival as compared with unsterilized soil.

2. *The Influence of Rates of Infestation, Soil Acidity, and Temperature on the Suppression of Pathogenicity by Soil Fungi*

It has been shown above that as the amount of *F. culmorum* inoculum added to unsterilized soil was increased the stand of healthy seedlings decreased. In a similar experiment conducted with sterilized soil infested with selected soil fungi, it was shown that as the rate of infestation with a

* Identified by Dr. K. B. Raper, Acting Head, Fermentation Division, Northern Regional Research Laboratory, Peoria, Ill.

2% cornmeal-sand inoculum of *F. culmorum* was increased from 1 to 2, 4, and 8%, the stands of seedlings in sterilized soil to which *Phialophora* had been added decreased from 45 to 35, 15, and 3%. There were indications of a similar effect of increased concentrations of inoculum in soil to which *Gliocladium* and *Acremonium* had been added, but the virulence of the *F. culmorum* inoculum was very high and suppressive effects of the soil fungi were obscured at a lower level of infestation with *F. culmorum* inoculum.

Experiments have shown that there may be considerable variation in the virulence of different preparations of inoculum. This observation was also made by Tyner (14). Since results vary with different rates of infestation the results of different experiments with the same isolates of fungi but with different preparations of inoculum would also be expected to vary. Greater increases in seedling stand resulted from the addition of *Phialophora* to sterilized soil infested with *F. culmorum* in experiments in which soil-oat hull medium was used to culture the fungi than when sand-cornmeal inoculum was used. Such differences have been attributed to a higher virulence of the sand-cornmeal inoculum, and hence a higher concentration of *F. culmorum* than was obtained by adding soil-oat hull inoculum to the soil. *Gliocladium* and *Acremonium* are less effective than *Phialophora* in reducing the incidence of blight caused by *F. culmorum*, and their effect is obscured with lower concentrations of *F. culmorum* in the soil, hence if the virulence of the *F. culmorum* inoculum were too high, or the amount added to sterilized soil were too great, no increase in seedling stand would be obtained by the addition of *Acremonium* or *Gliocladium* to the soil.

Different soils and mixtures of soils were sterilized, then the pH values were determined with a Leeds Northrup glass electrode pH metre. The pH values were as follows: a muck type of soil, 7.6; a mixture of loam and sand, 7.2; a mixture of loam and peat moss, 6.8; and peat moss, 5.2. The stands of brome grass seedlings obtained when these soils were not infested did not vary significantly. Similarly the stands were low in all soils infested with *F. culmorum* with no significant differences between the soils. However, in soils infested with *F. culmorum* and *Phialophora* the stands were as high as 100% in the muck soil of pH 7.6 and as low as 30% in the peat moss of pH 5.2, while in the soils with intermediate pH values, the stands were intermediate. *Phialophora* was therefore more effective in suppressing pathogenicity in the more alkaline than in the acid soils. Although the influence of soil reaction on *Acremonium* and *Gliocladium* was not as striking as that on *Phialophora* in this experiment, because of low stands in all soils, these fungi also suppressed the pathogenicity of *F. culmorum* most in the alkaline soils. *Trichoderma* and *Penicillium* were not effective in any of the soils.

The influence of soil temperatures on the antagonistic effects of the five selected isolates was compared. Wisconsin temperature tanks were used to regulate the soils at temperatures of 15°, 20°, 25°, 30°, and 35° C. One can in each tank contained unsterilized loam soil infested with 2% of *F. culmorum* inoculum. All the other cans contained sterilized soil, and all but one in each

tank was infested with 2% of *F. culmorum* inoculum. To individual cans of soil thus infested, 2% of the soil-oat hull inoculum of the soil fungi was added. The infested soil in one can was not infested with a soil fungus. One hundred brome grass seeds were sown in each can and the soil moisture was held at about 40% of the moisture holding capacity. The stands of seedlings in each soil at the various temperatures are presented in graph form in Fig. 8.

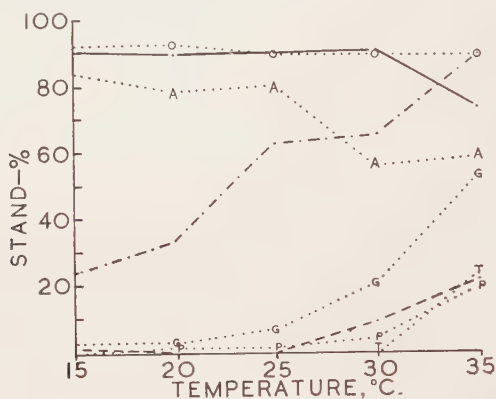


FIG. 8. The relation of temperature to the stands of brome grass seedlings in soil infested with *F. culmorum* and certain soil fungi. — Sterilized soil, uninfested. --- Sterilized soil, infested with *F. culmorum*. Sterilized soil infested with *F. culmorum* and a soil fungus. A = *Acremonium*. O = *Phialophora*. G = *Gliocladium*. P = *Penicillium*. T = *Trichoderma*. -.-.- Unsterilized soil infested with *F. culmorum*.

The stands in sterilized soil infested with *F. culmorum* were 1 to 0% at temperatures of 15° to 25° C., but increased to 9% at 30°, and to 21% at 35° C. Neither *Penicillium* nor *Trichoderma* caused any increase in stand at any temperature whereas *Phialophora* increased the stand to at least the equal of that in uninfested sterilized soil at all temperatures. *Acremonium* caused a greater increase in stand at the lower than at the higher temperatures, while *Gliocladium* on the other hand, was most effective at the higher temperatures. In unsterilized soil infested with *F. culmorum* the stands were low at the lower temperatures but increased significantly toward higher temperatures. These antagonistic effects to *F. culmorum* in unsterilized soil show a similar relation to temperature in this experiment as has been illustrated in experiments described above, but in comparing this with the various responses to temperature of the individual competitive fungi, it is interesting to note that the antagonistic effects of *Phialophora* were not appreciably influenced by soil temperature, while those of *Acremonium* were greatest at the lower, and those of *Gliocladium* greatest at the higher temperatures.

3. The Relation Between the Suppression of *Fusarium* Blight by Certain Soil Fungi and Their Antagonistic Effects in Culture

The influence of the soil fungi on the growth of *F. culmorum* in culture was investigated in comparison with their influence on seedling blight as shown above.

In one experiment the isolates were grown in apposition with *F. culmorum* on potato dextrose agar and on a soil - oat hull medium in Petri dishes, the inocula being placed about 3 cm. apart. The association effects and relative growth of *F. culmorum* and different soil fungi varied. Neither *Phialophora* nor *Acremonium* made very extensive growth on agar for *F. culmorum* grew more rapidly and soon surrounded the colonies of the former fungi. On the soil medium, however, *Phialophora* grew more rapidly than and suppressed the growth of *F. culmorum*, while the growth of *Acremonium* was about the same on the soil as on the agar medium. *Gliocladium* produced colonies of limited size on both media while *F. culmorum* covered the remainder of the dish. On agar, however, *F. culmorum* grew close to but not in contact with *Gliocladium*. A similar but more pronounced effect was demonstrated by *Penicillium*, which produced a distinct zone of inhibition on agar, but on the soil medium *F. culmorum* grew in close contact with it. *Trichoderma* very effectively suppressed *Fusarium* development on agar for it quickly covered the medium and limited the development of the pathogen to a very small colony with limited aerial mycelium. On the soil medium *F. culmorum* developed more abundantly than *Trichoderma* and confined the growth of the latter to a sector of the medium. These results show that the association effects of these soil fungi and *F. culmorum* differ on different media. Furthermore the fungi that effectively reduced disease incidence were less effective in suppressing the pathogen on agar than other fungi that did not reduce the amount of blight.

In addition to the above experiments the relative growth of *F. culmorum* and the soil fungi was compared on two solid media and a liquid medium inoculated with mixed spore suspensions of the fungi. The two solid media were potato dextrose agar and the soil - oat hull medium described above. The liquid medium was a potato dextrose decoction. Spore suspensions in sterile water were made from the various fungi. An equal volume of a suspension of *F. culmorum* conidia was added to the spore suspension of each soil isolate. Petri dishes of the two solid media and 200 cc. Erlenmeyer flasks containing 25 cc. of the liquid medium were inoculated with the mixed suspensions. The resultant growth of *F. culmorum* and the soil fungi was similar on all media. *F. culmorum* and *Phialophora* made about equal growth, hence neither seemed to suppress the other very strongly if at all. *Fusarium* made only slight growth when mixed with *Gliocladium*, and no growth at all when mixed with *Trichoderma* and *Penicillium* while the latter fungi covered the entire medium in every instance. These results, like those on growth in apposition, show that *Phialophora*, which greatly reduces the pathogenicity of *F. culmorum*, does not effectively suppress its growth in culture, while other fungi that have less effect or no effect on pathogenicity strongly suppress growth in culture.

Additional experiments showed that when these soil fungi were grown on potato dextrose decoction for seven days, the filtrates of the media on which *Gliocladium*, *Trichoderma*, or *Penicillium* had grown were toxic to *F. culmorum*.

When nutrients were added, *Fusarium* grew abundantly on the filtrates from *Phialophora* and *Acremonium*, moderately on the filtrates of *Gliocladium* and *Trichoderma*, and not at all on the *Penicillium* filtrate. *F. culmorum* inoculum was soaked in filtrates of the various fungi before it was mixed in the soil. The filtrates of *Gliocladium* and *Penicillium* were the only ones that reduced the virulence of the inoculum, and of these *Penicillium* was the more effective. From the results it is concluded that the production of toxic filtrates by these soil fungi does not correlate with the ability of the fungi to suppress the seedling blight caused by *F. culmorum*.

4. The Relation Between the Suppression of the Incidence of Blight by Certain Soil Fungi and the Suppression of *F. culmorum* Development in the Soil

Experiments were conducted to compare the influence of antagonistic soil fungi on the development of *F. culmorum* in the soil with their influence on the incidence of seedling blight. Sterilized soil was infested with *F. culmorum* soil - oat hull inoculum, and quantities of this infested soil sufficient for two 5 in. pots each were infested with *Phialophora*, *Acremonium*, *Gliocladium*, *Penicillium*, and *Trichoderma* isolates, while soil for two other pots was not further infested. Two pots of unsterilized soil were also infested with *F. culmorum* inoculum. Brome grass seeds were sown at the rate of 25 per pot and the resulting stands were counted two weeks later. The numbers of *F. culmorum* isolates per gram of each soil were also counted by means of the dilution plate technique.

The results (Table V) show that, with the exception of *Penicillium* and *Trichoderma*, the fungi tested significantly increased the stands of seedlings in infested soil. The extent of this increase was not related directly to the

TABLE V

THE INFLUENCE OF CERTAIN SOIL FUNGI ON THE STAND OF SEEDLINGS AND THE NUMBER OF *F. culmorum* ISOLATES IN INFESTED SOIL

Soil	Fungus added	Stand, %	<i>F. culmorum</i> isolates per gram
Unsterilized	—	52	33,000
Sterilized	—	4	94,000
"	<i>Phialophora</i>	82	71,000
"	<i>Acremonium</i>	54	76,000
"	<i>Gliocladium</i>	36	94,000
"	<i>Penicillium</i>	10	(<i>Penicillium</i> covered plate)
"	<i>Trichoderma</i>	2	(<i>Trichoderma</i> covered plate)

concentration of *F. culmorum*. In sterilized soil infested with *F. culmorum* the stand was low and the number of *F. culmorum* isolates was high, but with the addition of *Gliocladium* to otherwise similarly infested soil the stand was strikingly increased but there was no change in the numbers of isolates of the pathogen. Further, the addition of *Phialophora* to infested soil caused a remarkable increase in stand but only a slight decrease in the concentration of

F. culmorum, whereas *Acremonium* caused a less marked increase in stand but almost as great a suppression of the pathogen. Or again, the stands in unsterilized soil and in *Acremonium* infested sterile soil were almost identical, but there were more than twice as many isolates of *F. culmorum* per gram in the latter than in the former soil. This strongly suggests, therefore, that although these soil micro-organisms influence the development of *F. culmorum* in the soil to a certain degree, they must exert a more effective suppressive influence on the pathogen in some phase more closely associated with the seedlings. The developmental phases of the seedling were consequently studied next.

By the dilution plate method the numbers of *F. culmorum* isolates in the rhizospheres, and as well in the soil apart from the roots of brome grass seedlings, were counted 10 days after the seeds were sown in sterilized loam soil infested with 1% of *F. culmorum* sand-cornmeal inoculum and 2% of the inoculum of different soil isolates. In Table VI the stands of seedlings and the numbers of isolates in the variously infested soils are shown. The concentration of the pathogen was consistently greater in the rhizosphere than in

TABLE VI

THE INFLUENCE OF SOIL FUNGI ON THE STAND OF SEEDLINGS AND ON THE NUMBERS OF *F. culmorum* ISOLATES FROM THE RHIZOSPHERE 10 DAYS AFTER SOWING IN INFESTED SOIL

Inoculum	Stand, %	<i>F. culmorum</i> isolates per gram	
		Rhizosphere	Soil apart from roots
Check	13	16,000	8800
<i>Phialophora</i>	80	13,800	7800
<i>Acremonium</i>	37	16,900	11,800
<i>Gliocladium</i>	33	18,000	12,000

the soil apart from the roots, even where there was little seedling blight, and there was no consistent correlation between seedling stand and the numbers of isolates of the pathogen either in the rhizosphere of 10-day seedlings or in the soil apart from the roots of the seedlings.

Since the above comparisons relate to a stage of seedling development considerably older than that at which most of the blight occurs, another experiment was conducted in which counts were made of the numbers of isolates associated with seedlings at the time of emergence. Brome grass seeds were sown in pots of sterilized soil infested with 2% *F. culmorum* inoculum and 2% of the inoculum of the different soil isolates as well as in pots of infested unsterilized soil. Five days later when the seedlings were just starting to emerge, they were removed from the soil and, after shaking off the loose soil, the seedlings with the remains of the seeds attached were put immediately into flasks of sterile water and dilutions were made for plating.

In duplicate pots the seedlings were allowed to grow so that the stands in the infested soils could be counted. These stands and the numbers of isolates of *F. culmorum* counted in the soil apart from and associated with germinating seeds are given in Table VII.

TABLE VII

THE INFLUENCE OF SOIL FUNGI ON THE STAND OF SEEDLINGS AND ON THE NUMBER OF *F. culmorum* ISOLATES IN THE SOIL CLOSELY ASSOCIATED WITH GERMINATING SEEDS IN INFESTED SOIL

Inoculum	Stand in duplicate pots, %	<i>F. culmorum</i> isolates per gram of soil	
		Spermatosphere	Apart from seeds
Check	0	80,000	13,300
<i>Phialophora</i>	79	10,200	7400
<i>Acremonium</i>	0	66,600	13,800
<i>Gliocladium</i>	8	31,400	11,700
Unsterilized soil	56	12,600	12,500

The stands varied from 0% in the check to 79% with the addition of *Phialophora*. The unusually small increase in stand brought about by *Gliocladium*, and the absence of any increase with the addition of *Acremonium* is attributed to too low a concentration of these fungi in relation to the concentration of *F. culmorum* in the soil. As in the previous experiments, there was no consistent correlation between the numbers of isolates in the soil and the stands of seedlings. The numbers of isolates in close association with the germinating seeds was in most instances much higher than in the surrounding soil, and these numbers were highest in the soils with the lowest stands, lowest in the soils with the highest stands, and intermediate in other soils. This zone, surrounding the germinating seed and developing under its influence a biological balance different from that of the soil farther removed, we designate the 'spermatosphere'. While there was a positive correlation between the numbers of *F. culmorum* isolates in the spermatosphere and the incidence of blight, the relation was not absolute for in this experiment *Acremonium* reduced the number of isolates in the soil but as in the check no seedlings survived. Also *Gliocladium* reduced the numbers of isolates to about 39% of the number in the check soil, but increased the stand to only 8%.

Microscopic examination of germinating seeds from sterilized soil infested with *F. culmorum* showed that hyphae of the pathogen grew abundantly on the hulls, on and in the endosperm, and attacked and killed the developing embryos. When *Phialophora* had been mixed with the infested soil the hyphae of this fungus were found growing in the hulls of the seeds while *F. culmorum* hyphae were less abundant on the hulls, scarce in the endosperms, and seldom were found in the embryos.

The above observation suggests that the hulls, which are the dead lemma and palea surrounding the caryopsis, provide a saprophytic substrate that may influence either the level of aggressiveness of the pathogen, the microbiological

balance between *F. culmorum* and other soil micro-organisms at that locus, or both of these. To test further these possibilities the hulls were removed from a quantity of seeds with forceps, and the stands resulting from sowing these dehulled seeds in soil infested with *F. culmorum* and antagonistic soil fungi were compared with the stands from normal seeds in the same soils. The results (Table VIII) showed that there were no significant differences in the stands from normal and dehulled seeds in uninfested sterilized and unsterilized soils, or in sterilized soil infested with *F. culmorum* alone. Nevertheless, the

TABLE VIII

THE EFFECT OF REMOVING THE HULLS OF BROME GRASS SEEDS ON THE STANDS IN VARIOUSLY INFESTED SOILS

Soil	Infestation	Stand, %	
		Normal seeds	Dehulled seeds
Sterilized	Check	92	90
"	<i>F. culmorum</i>	0	3
"	<i>F. culmorum</i> + <i>Phialophora</i>	45	24
"	<i>F. culmorum</i> + <i>Gliocladium</i>	7	22
Unsterilized	Check	83	86
"	<i>F. culmorum</i>	17	60

stand in infested soil to which *Phialophora* was added was lower from dehulled than from normal seeds. The reverse relation was shown for *Gliocladium* and unsterilized soil, for in these instances the removal of the hulls caused the stand to be significantly increased.

Evidence is thus presented that in the absence of effective competition *F. culmorum* may readily colonize the hulls and proceed to make a virulent attack on the germinating seed. However, in the presence of other soil micro-organisms the hulls may be colonized by some of these organisms as well as by *F. culmorum*. The hulls may therefore be decisive in determining the microbiological balance in this strategic locus and thereby influence the attack of the seedling by a pathogen present in the soil.

D. Variations in Microbiological Antagonism in Different Field Soils

During the summer of 1944, one sample of clay and 15 samples of loam soil differing in cultural history were collected from fields near St. Catharines and Vineland, Ont. Four 5 in. pots were filled with each soil, two of which were infested with 4% of *F. culmorum* inoculum, and one day later 25 seeds of crested wheat grass were sown in each pot. When no inoculum was added to these soils the stands of seedlings (Table IX) with three exceptions varied only from 70 to 78%; in a loam soil from a field of alfalfa and timothy and in loam from a field in which alfalfa had grown for a number of years followed by corn, it was as high as 84%, while in loam from grass sod the stand was only 64%. When these soils were uniformly infested with *F. culmorum*, the stands varied

TABLE IX

PERCENTAGE STAND FOLLOWING EQUAL INFESTATION WITH *F. culmorum* OF DIFFERENT FIELD SOILS COLLECTED NEAR VINELAND AND ST. CATHARINES, ONT.

Sample number	Soil texture	Cultural history	Uninfested soil	Infested soil
1	Clay	Alfalfa and timothy mixture	72	16
2	Loam	Alfalfa and timothy mixture	84	72
3	"	Oats after other cereals	78	36
4	"	Grass (in fence corner)	72	62
5	"	Grass (roadside)	76	76
6	"	Grass (pasture)	74	74
7	"	Grass (roadside)	72	66
8	"	Grass sod (black)	64	58
9	"	Grass sod (brown)	74	42
10	"	Wheat after other cereals	70	58
11	"	Corn after alfalfa	84	48
12	"	Old stand alfalfa and timothy (timothy killed)	74	40
13	"	Old stand alfalfa and timothy (alfalfa killed)	72	58
14	"	Peach orchard	78	56
15	"	Wooded area (under sparse growth of trees)	78	48
16	"	Wooded area (under dense growth of trees)	70	26

from 16 to 76%. The highest stands were in loam soils that had grown grass or an alfalfa-grass mixture for a considerable period of time. The lowest stands were in clay, also from a field growing alfalfa and grass, and in loam from a wooded area. Intermediate stands were generally obtained in soil from cultivated fields.

In October of the same year quantities of the first four soils listed in Table IX were collected for further experimentation. The clay and loam soils from the same field of alfalfa and timothy were selected because, while they had identical cultural histories, *F. culmorum* inoculum caused more seedling blight in the clay than in the loam. The other two soils were selected because one had grown cereal crops for a number of years, while the other had grown grass without being cultivated for many years. Hence these four soils gave contrasts in texture and cultural history. Fifty brome grass seeds were sown in each of four 6 in. pots of each soil infested with 4% *F. culmorum* inoculum by weight, and in two pots of each soil not infested. The stands were counted two weeks later. From two experiments using three Petri dishes for each dilution, counts were made of the numbers of bacteria, *F. culmorum* isolates, and other fungi per gram in the infested soil. The stands in uninfested and infested soils, and the numbers of soil fungi and bacteria as well as the numbers of *F. culmorum* isolates in the infested soils are given in Table X.

The stands in the uninfested soils did not vary more than 8% whereas, in the infested soils, it was again significantly lower in the clay than in loam from the same field. There was little variation in the stands among the loam soils of different cultural history. If seedling blight were a function simply of the development of *F. culmorum* in the soil, the numbers of isolates should be higher in the infested soils with the lower stands. The number of isolates of

TABLE X

RELATION BETWEEN SEEDLING STANDS AND THE NUMBERS OF BACTERIA, FUNGI, AND ISOLATES OF THE PATHOGEN IN FOUR VINELAND SOILS EQUALLY INFESTED WITH *F. culmorum*

Texture	Soil Cultural history	Stand, %		Micro-organisms, thousands per gram		
		Uninfested	Infested	<i>F. culmorum</i> isolates	Other fungi	Bacteria
Clay	Alfalfa and timothy	75	22.5	123	300	17,200
Loam	Alfalfa and timothy	80	41.5	101	280	16,900
"	Oats	72	40.0	110	270	57,900
"	Grass	72	37.2	48	320	17,000

the pathogen was 18°C higher in the clay than in the loam from the same field, hence in this instance the numbers were highest in the soil with the most seedling blight. The numbers in the three loam soils, however, varied greatly without relation to seedling stand. Similarly if suppression of blight in these soils were a function of the numbers of fungi and bacteria populating them, these organisms should have been lowest in the clay, but such was not the case. There was no evident correlation between differences in numbers of fungi or bacteria and differences in seedling blight.

Results of temperature experiments with these soils (13) showed that seedling blight was more severe at all temperatures in the clay than in the loam soils. There was also a correlation between the numbers of *F. culmorum* isolates and seedling blight at the higher (20° to 35° C.) but not at the lower soil temperatures (10° to 15° C.). In agreement with the results given in Table X there was no consistent relation between numbers of soil fungi or bacteria in the soils and seedling blight when infested with *F. culmorum*.

A similar comparison of the stands of grass seedlings in field soils infested with 6% *F. culmorum* was made with soils collected near Harrow, Ont., which varied in texture from sand to clay. All had grown cereal or forage crops for a number of years. The resulting stands two weeks after sowing are given in Table XI. In the uninfested soils the stands varied little, but among the infested soils they varied from 10 to 54.5%. The lowest stands were in clay, that in clay loam somewhat higher, those in loam still higher, while the highest stands of all were in sandy soils. The stands were low in all four infested sterilized soils with little variation among them. However, in contrast with these results, in the same soils unsterilized and infested, the stands were slightly higher in the clay than in the lighter textured soils when they were sterilized before infestation. This suggests that the differences between the stands in the unsterilized field soils uniformly infested with *F. culmorum* is due to the influence of texture on the microbiological balance and the effect of this on the pathogen.

The numbers of fungi, bacteria, and actinomycetes per gram in two of the clays, a loam and a sandy soil (Table XI : Nos. 1, 2, 6, and 8), were counted by

TABLE XI

PERCENTAGE STAND OF BROME GRASS IN HARROW SOILS UNIFORMLY
INFESTED WITH *F. culmorum*

Sample number	Soil texture	Last crop	Unsterilized soil		Sterilized soil infested with 3% <i>F. culmorum</i>
			Uninfested	Infested with 6% <i>F. culmorum</i>	
1	Clay	Timothy	85	10.0	9.0
2	Clay	Wheat	86	11.5	4.0
3	Clay	Oats	88	13.5	—
4	Clay loam	Alfalfa	87	19.5	—
5	Loam	Oats	91	41.5	—
6	Black loam	Oats	90	46.5	3.5
7	Sand	Grass	91	54.0	—
8	Sand	Oat stubble	86	54.5	3.5

the dilution plate method to investigate any possible correlation that might exist between numbers of micro-organisms and suppression of seedling blight in these soils. In addition the numbers of these organisms in the rhizospheres of brome grass seedlings were investigated. For these counts dilutions were made of the soil apart from the roots and as well of the rhizosphere soil of 10 day old seedlings. Dilutions of 1 : 10,000 for fungus counts were plated with peptone-glucose agar, Czapek's agar modified by reducing the sucrose content to 1%, and brome grass seed extract agar. The seed extract was prepared by autoclaving 200 gm. of brome grass seeds in 1000 cc. of water for 15 min. at 15 lb. pressure, filtering the liquid, and restoring to the original volume with water. Portions (200 cc.) of this extract were transferred to 300 cc. Erlenmeyer flasks, to each of which were added 0.4 gm. of sucrose and 5 gm. of agar. The flasks were plugged with cotton and sterilized. To each flask of this medium, as to flasks containing similar quantities of peptone-glucose and Czapek's agars, 1 cc. of normal sulphuric acid was added after the media were melted in preparation for pouring in the Petri dishes containing 1 cc. of the required dilutions of soils. Dilutions of 1 : 100,000 were plated in Petri dishes with sodium albuminate and soil extract agars (3) for counts of bacteria. The actinomycete colonies that could be distinguished from the bacteria were also counted on these plates.

In Table XII are given the stands of seedlings in the four soils infested with 6% *F. culmorum* inoculum. The numbers of fungi, bacteria, and actinomycetes in the rhizosphere of seedlings as well as in the soil apart from the roots in pots of the same soils not infested with *F. culmorum* are also given. The numbers of fungi are the averages from three Petri dishes of each of the three acidified agars, and the numbers of bacteria and actinomycetes are the averages from three Petri dishes of albuminate and two of soil extract agar. The results show that the stands were lowest in the two clays, much higher in the loam, and highest of all in the sand. There was no consistent correlation between the numbers of actinomycetes in the soil and the stands of seedlings,

TABLE XII

THE NUMBERS OF MICRO-ORGANISMS IN THE RHIZOSPHERE AND IN THE SOIL APART FROM THE ROOTS OF SEEDLINGS 10 DAYS AFTER EMERGENCE IN DIFFERENT FIELD SOILS AS COMPARED WITH THE STANDS WHEN THE SOILS WERE INFESTED WITH 6% *F. culmorum* INOCULUM

Soil	Stand when infested, %	Numbers in thousands per gram					
		Rhizosphere			Soil apart from the roots		
		Fungi	Bacteria	Actino- mycetes	Fungi	Bacteria	Actino- mycetes
Clay (oats)	15	271	70,594	3511	205	37100	2125
Clay (timothy)	10	583	94,438	1532	240	39680	1625
Loam (oats)	43	346	120,746	3200	180	26354	2347
Sand (oats)	51	200	109,000	2531	149	21318	2439

whereas both bacteria and fungi were more abundant in the clays than in the loam or sand. This is contrary to what would be expected if the suppression of the pathogenicity of *F. culmorum* were a function of the numbers of these micro-organisms, for the stands were the lowest and blight most severe in the clay soils. In the rhizospheres of seedlings all three groups of micro-organisms, and especially the bacteria, were more abundant than in the surrounding soil. There was no consistent relation between the numbers of fungi or actinomycetes and stands, but bacteria were less abundant in the rhizospheres in the clay than in the loam and sandy soils.

Another experiment was carried out with the same soils, but this time, five days after sowing, the seedlings, which were just about to emerge, were removed from the soil, washed in sterile water, and further dilutions were made for plating. Counts were also made of the micro-organisms in the surrounding soil. Since the soils had been kept moist for considerably longer than for the preceding experiment, the results of the two experiments are not quantitatively comparable. The stands of seedlings in duplicate pots of these soils infested with *F. culmorum* are given in Table XIII along with the numbers of fungi, bacteria, and actinomycetes in the soil closely associated with the germinating seeds and in the surrounding soil.

As in the previous experiment the stands were lower in the two clay soils than in the loam and sandy soils equally infested with *F. culmorum*. The numbers of fungi were higher in the spermatosphere than in the surrounding soil, but, as was the case with the rhizospheres of 10-day-old seedlings there was no consistent relation between these numbers of fungi and the stands in the infested soils. The numbers of actinomycetes were actually lower in the soil associated with the germinating seeds than in the surrounding soil, and there was no consistent relation with stands. The numbers of bacteria, however, were from 233% to 830% higher in the spermatosphere than in the surrounding soil and were much higher in association with seeds germinating in sand and loam than in clay soils. Hence there is a correlation between

TABLE XIII

NUMBERS OF MICRO-ORGANISMS IN THE SOIL APART FROM AND ASSOCIATED WITH GERMINATING SEEDS FIVE DAYS AFTER SOWING IN DIFFERENT FIELD SOILS, AS COMPARED WITH THE STANDS WHEN THE SOILS WERE INFESTED WITH 6% *F. culmorum*

Soil	Stand when infested, %	Numbers in thousands per gram of soil					
		Spermatosphere			Apart from the seedlings		
		Fungi	Bacteria	Actino- mycetes	Fungi	Bacteria	Actino- mycetes
Clay (oats)	23	253	289,164	631	219	86,721	1559
Clay (timothy)	32	350	339,246	246	268	62,716	692
Loam (oats)	67	415	437,854	242	215	78,157	2806
Sand (oats)	74	253	424,124	744	121	45,583	1322

these greatly increased numbers of bacteria and the stands of seedlings obtained when these soils were infested with *F. culmorum*.

Discussion

Most of the fungi that have been shown in these studies to be pathogenic to brome and crested wheat grass seedlings have also been reported as pathogens of cereal grasses in Canada (2). In recent years it has been shown repeatedly that many of the soil-borne pathogens of cereals are also destructive to forage grasses (4, 10, 11, 12, 15, 16). In addition it was shown in these studies that pathogenic *Helminthosporium* and *Fusarium* isolates were carried on brome and crested wheat grass seeds. The carrying of such pathogens on seeds is also an important consideration in seedling blight and root rots of cereals (1, 5, 6). Such close parallels in the seedling blight and root diseases of the cereal and forage grasses stress the need for considering, from a disease standpoint, the relations of forage grasses in crop rotations including cereals.

Although the cultivation of susceptible crops may have an important influence on the prevalence of seedling pathogens in the soil, other factors are also of major importance. The studies of other workers (7, 8, 9) have shown that soil micro-organisms influence the virulence of fungi that cause root rots of wheat. The studies reported here have considered the influence of soil micro-organisms on the virulence of *F. culmorum* to grass seedlings, and on its development in the soil.

The addition of 1% of *F. culmorum* inoculum was as effective as larger amounts in causing severe seedling blight in sterilized soil. In unsterilized soil, the incidence of blight increased as the amount of inoculum added to the soil was increased, but even 6% of inoculum caused less blight than did 1% in sterilized soil. If the concentration of the pathogen in the soil is decisive in determining disease incidence the above results can be explained on the basis of the rapid increase of the pathogen to a high concentration in sterilized soil as contrasted with the less striking increase in unsterilized soil. Hence,

within wide limits, the initial concentration of inoculum in sterilized soil is of little importance because of the rapidity with which it increases, whereas the initial concentration in unsterilized soil is important in determining the resulting incidence of blight because the increase is held in check by the other micro-organisms in the soil.

In sterilized soil the effect of temperature on blight appeared to be the result of its effect on the development of the pathogen in the soil. Further support for the view that seedling blight depends to a large extent on the concentration of the pathogen was obtained from experiments that indicated that with increased rates of infestation of sterilized soil, the effect of temperature on blight could be largely obscured and blight become equally severe at all temperatures. In unsterilized soil the relation of temperature to the development of *F. culmorum* was influenced by soil micro-organisms. These suppressed the pathogen most at the temperatures that were optimum in sterilized soil, so that its maximum development in the unsterilized soil was at significantly lower temperatures. The optimum temperature for blight was also lower in unsterilized than in sterilized soil. Again the evidence indicates that the incidence of blight is a function of the concentration of the pathogen in the soil. The effect of temperature and micro-organisms on blight is apparently the result of their influence on the development of *F. culmorum* in the soil.

The differences in the antagonistic effects of selected soil fungi and of unsterilized soil offered a suitable opportunity of comparing and contrasting their relative influences on *F. culmorum* development and on the incidence of blight. Such comparisons showed that there was no consistent correlation between their effect in suppressing the development of the pathogen in the soil and their effect in suppressing blight. However, the development of the pathogen in the environment in the immediate vicinity of the germinating seeds was different from that in the surrounding soil and did vary with disease incidence. This locus, in which the germinating seed induces a characteristic microbiological balance is designated as the 'spermatosphere'. Furthermore, in different field soils the biological balance in this spermatosphere was shown to be different although in all of them the numbers of bacteria in the spermatosphere were correlated with the suppression of blight, while the numbers in soil apart from the seedlings were not.

There are, therefore, two soil environments in which the microbiological balance has an important bearing on seedling blight. The microbiological balance in the general soil environment apart from the seedlings is of primary importance in that it must contain the pathogen in an effective concentration. The microbiological balance in the other environment in the immediate vicinity of the germinating seeds, the spermatosphere, depends on that of the surrounding soil but is very different from it. The resulting development in the spermatosphere is of major importance in determining the attack on the young seedling by a pathogen existing in the soil.

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HEART ROT OF OAKS CAUSED BY *POLYPORUS OBTUSUS*¹

By C. G. RILEY²

Abstract

Polyporus obtusus Berk. is a heartwood decaying fungus that attacks several American species of broadleaved trees, principally oaks. It has a wide geographical range, but tends to occur in localized concentrations within which it causes great destruction, while elsewhere it is rarely found. On account of its peculiar distribution, *P. obtusus* is not well known, and the published references to it are comparatively few. A local concentration of this disease, the only one known in Canada, occurs at the Petawawa Forest Experiment Station, Ont. Here, oak trees from 0.8 to 4.8 in. in diameter were being attacked. The resulting white rot continues to advance into the sapwood, ultimately causing death of the tree. The large, cream-coloured, bracket-like sporophores are borne on the diseased trees in great abundance in some seasons, and are comparatively scarce in others.

Polyporus obtusus Berk. is perhaps the least known of all American polypores of comparable geographical range and destructive capacity. Published references are strikingly few. What appears to be the only original general account of the disease caused by this fungus, was published by Spaulding (8) in 1905. Von Schrenk and Spaulding (7) dealt with it again in 1909, and Lorenz and Christensen (2) devoted a brief paragraph to it in 1937. Other references are primarily taxonomic, or are simply records of occurrence. The species has been omitted frequently from systematic accounts of the polypores, even where these deal with regions within which it is known to occur. These circumstances seem to suggest that *P. obtusus* is an unimportant pathogen of rare occurrence; indeed, it is noted as "rare" by some authors. Actually, however, it is very widely distributed in North America, and occurs on a number of deciduous tree hosts, principally oaks. In a personal letter, dated February 16, 1946, the late Dr. L. O. Overholts of Pennsylvania State College kindly provided the following information for the purpose of this publication. In his collection are specimens of *P. obtusus* from Pennsylvania, New Jersey, Maryland, District of Columbia, Virginia, North Carolina, Georgia, Florida, Alabama, Louisiana, Mississippi, Tennessee, Wisconsin, Minnesota, Iowa, Missouri, Arkansas, Oklahoma, Kansas, Arizona, New Mexico, Oregon, and Ontario. The species has also been recorded at Berthierville, Que. (6). These records greatly enlarge the previously published geographical range of the fungus. Regarding hosts, Dr. Overholts stated that he had or knew of reliable records of the occurrence of *P. obtusus* on *Quercus*, *Acer*, *Juglans*, *Liquidambar*, *Fagus*, *Robinia*, *Pyrus*, and *Carya*. Add to the foregoing the published facts that "the disease is quite destructive locally to several species of oak trees" (8), and "a large number of trees are usually found affected in a locality" (7), and the situation becomes somewhat

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of a puzzle. Actually, it appears that the fungus occurs in dense concentrations in widely separated localities within which it is a vigorous and destructive pathogen, but outside of which it is of comparatively rare occurrence.

The Ontario record mentioned above refers to a dense, local concentration of *P. obtusus* at the Petawawa Forest Experiment Station, about 95 direct miles northwest of Ottawa. Here the principal host is red oak (*Quercus borealis* Michx.), though white oak (*Q. alba* L.) and bur oak (*Q. macrocarpa* Michx.) are also attacked. Most of the observed infected trees are on poor sites for these species, principally on rocky ridges, and associated with aspen, jack pine, and red maple.

A 1/10 ac. sample plot was examined in a locality where sporophores of *P. obtusus* were particularly abundant. The plot contained 61 red, and 12 white oaks, many of which occurred as coppice clusters. They ranged in age from 15 to 25 years, were 0.8 to 4.8 in. in diameter at breast height, and 8 to 29 ft. high. Heartwood decay, typical of that caused by *P. obtusus*, was found in 57 of the red, and in 3 of the white oaks. The remaining 13 trees were all among the youngest and smallest of those examined. Sporophores of the fungus were associated with the decay in 11 of the red oaks and all three of the infected white oaks. Some sporophore-bearing bur oaks were found nearby.

The nature of the disease and the appearance of the affected wood on the Petawawa plot were essentially as described in the references already cited. The fungus appeared to have gained entrance through various kinds of wounds such as frost cracks, mechanical abrasions, and branch snags. It is of interest to note that Spaulding (8) found the disease consistently associated with insect tunnels. Some badly infected trees exhibited no external symptoms or wounds to suggest that they were diseased. In some trees, the decay occurred only in the lower trunk, and in others, only in the crown.

In its early stage, the decay appears as creamy-white concentric lines in cross section, or streaks in longitudinal section, within the early wood of the annual rings (Fig. 5). Badly decayed wood is spongy, and creamy white when freshly exposed, tending toward tan on drying. In cross-section, a

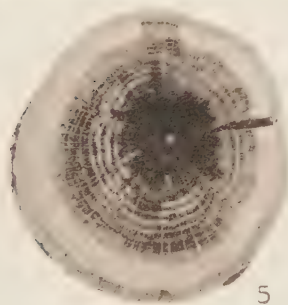
FIG. 1. Sporophore of *Polyporus obtusus* growing from an open knot on living red oak. (Diameter of host at top of section, 3 in.)

FIG. 2. Longitudinal section through specimen shown in Fig. 1. Note the structure of the sporophore, and the advanced decay surrounded by dark-stained heartwood.

FIG. 3. Sporophore of *Polyporus obtusus* growing from an old wound in living white oak. (Half natural size.)

FIG. 4. The ultimate fate of the host. A living red oak about 4.5 in. in diameter, badly decayed by *Polyporus obtusus*, and broken at the weakened point.

FIGS. 5, 6, and 7. *Polyporus obtusus* decay in a living red oak main stem at a height of between 4 and 5 ft. (diameter 2.4 in.). Fig. 5 shows the condition near the upper extremity of the advanced decay. Note the decay confined to the early wood of the annual rings. The remaining heartwood is darkly stained. Fig. 6 shows the condition at a point 2.5 in. below that shown in Fig. 5, while Fig. 7 is from 5.5 in. lower than Fig. 6. Note in Fig. 7 the sharply defined margin of the densely stained wood in contact with the healthy sapwood.



core of advanced decay may be delimited by a narrow dark line, or the entire remaining heartwood region may be abnormally dark (Fig. 6). Frequently, this discoloration becomes more intense toward the periphery. It may terminate abruptly in a dark line adjacent to the sapwood (Fig. 7). Longitudinally, the region of advanced decay may terminate rather abruptly (Figs. 5 to 7), or it may continue for a considerable distance, tapering to thin central or excentric streaks that sometimes extend into the branches. Typically, the dark-stained wood that surrounds the rot extends longitudinally beyond the decay region as a central core far up the stem and into the branches. Numerous tests were made by means of agar cultures, to determine whether *P. obtusus* was present in this dark-stained wood. The results were all either negative or inconclusive. Several organisms developed in the cultures, but *P. obtusus* was not found. Also, this fungus could not be obtained in culture from the dark-stained wood laterally surrounding the advanced decay, although it could be isolated readily from the margin of the decayed region. The cultural characteristics of the fungus have been described by Davidson, Campbell, and Vaughn (1). As the fungus advances within the infected tree, the decayed core continues to enlarge until the adjacent sapwood region is entirely involved and the affected branch or stem either dies or breaks over at the weakened point (Fig. 4).

Sporophores commonly occur at branch snags or open knots (Figs. 1, 2), at wounds (Fig. 3), and at the point of breakage (Fig. 4). They have been described in detail by Lowe (3), Overholts (4, 5), and others. They are conspicuous in the woods by reason of their size and colour (Fig. 4), being commonly 2.5 to 5.0 in. broad and 1.5 to 2.5 in. thick. They are firm, strongly convex, and bracket-like. The velvety to coarsely tomentose upper surface is creamy white to grayish, while the pore surface tends toward a darker cream or tan. The whole may become dark tan on drying. The tubes are coarse, with irregular mouths, and are commonly 0.5 to 1.0 in. long. The annual sporophores may occur in great abundance in one season, and be quite scarce in the same locality another year.

It is stated by Lorenz and Christensen (2) that *P. obtusus* is responsible for considerable losses in oak in the Central States. In Ontario, only the three species of oak already mentioned extend as far north as the Petawawa region, and there they do not form a commercially important constituent of the forest. Red oak is cut for sawlogs on the better sites, and here it is characteristically sound. Although no data are available to indicate the amount of loss caused by *P. obtusus*, the disease is not believed to be of great economic importance in Ontario at present. Further investigation in this respect is required. In the light of present knowledge, the most interesting feature of the disease lies in the peculiarity of its local and geographical distribution.

Acknowledgments

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